

Title	Perspectives on third generation gaseous biofuel
Authors	Guneratnam, Amita Jacob
Publication date	2017
Original Citation	Guneratnam, A. J. 2017. Perspectives on third generation gaseous biofuel. PhD Thesis, University College Cork.
Type of publication	Doctoral thesis
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Download date	2023-05-05 00:09:03
Item downloaded from	http://hdl.handle.net/10468/5458



Energy Engineering
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Perspectives on third generation gaseous biofuel
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Thesis submitted for the degree of Doctor of Philosophy to the National University of
Ireland, Cork

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December 2017

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Declaration

I hereby declare that this thesis is my own work and that it has not been submitted for another degree, either at University College Cork or elsewhere. Where other sources of information have been used, they have been acknowledged.

Signature:

Date: December 2017

Acknowledgements

This thesis would not have been possible without the support of the following people.

My supervisor, Professor Jerry D Murphy, for his constant guidance and encouragement.

My mentor Dr. Ao Xia for his enthusiasm, advice and support.

My second supervisor Dr. Vikram Pakrashi for his valuable suggestions and comments

Science Foundation Ireland, Ervia and Gas Networks Ireland for funding my work through the MaREI centre.

My colleagues at the Bioenergy Research Group and Environmental Research Institute for their guidance, friendship and support.

My parents and siblings for keeping me motivated throughout this journey.

My loving husband and in-laws for providing me with support and care.

Abstract

The study of third generation gaseous biofuels has been steadily increasing with innovation and research for high biomass yielding feedstock preferably that do not compete with food production or land usage. Government policies have also been instrumental in scoping for new feedstock that cannot be categorised as conventional biomass such as first and second-generation biofuels. Sugar or starch based crops, oil crops form first generation feedstock that can also be used as food or feed thus competing for land and food thus causing an increase in food prices. Ligno-cellulosic biomass that are hard to ferment and need pre-treatment for ease of process operation are considered as second-generation feedstock. Advanced biofuels that have high productivity such as micro and macro algae or certain plant based waste biomass are considered as third generation biofuel. Algal biomass can also be produced using waste streams such as CO₂ from industries (for producing micro-algae) and fish waste (for macro-algae). Hydrogen produced using surplus electricity can be then transformed to methane by biological or catalytic methanation is also considered as a candidate for third generation feedstock to produce gaseous biofuel. EU allows a weighting of 2 to third generation biofuels in assessing 2020 renewable energy in transport targets: these include for biofuels produced from algae and gaseous fuel from non-biological sources (hydrogen from surplus electricity).

Micro-algal biomass can be produced using waste exhaust streams from industries containing CO₂, SO_x, NO_x. One of the primary sources of fossil fuel such as coal is used for electricity generation. The emissions from a 1 GW_e coal power plant (operating at 35% electrical efficiency and a capacity factor of 75% can produce 6.72 million tonnes of CO₂ per annum) if captured can produce 2.69Mt of micro-algal (volatile solids) in a closed cultivation system with a carbon capture efficiency of 80 %, in a foot print of 19,200 hectare for a tubular photo-bioreactor. If this waste derived micro-algal biomass is subjected to a three-stage process of

dark fermentation, photo-fermentation and anaerobic digestion, ca.35 % of the primary energy in coal can be retrieved as renewable gaseous fuel. However, at the current state of technology, operating a tubular photo bioreactor is very expensive and thus the energy input to the cultivation system can be greater than energy output.

Another form of algal biomass such as macro-algae (seaweed) can also be produced by sequestering nitrogen from waste streams that are released by fish farms that can cause eutrophication of water. As global fish demand is increasing natural stocks will not be sufficient to cater to this demand, hence aquaculture will be contributing heavily to this supply demand gap. An integrated multi-trophic aquaculture system can be used around fish farms that sequester waste through co-culture of seaweed and mussels. A production of 168Mt of seaweed integrated with 13Mt of farmed salmon is required if 1.25 % of energy in transport is to be provided by seaweed biomass. However this involves operating 2600 anaerobic digesters, each treating 64,500t/a of *S.latisma* in coastal digesters.

Brown seaweed such as *Laminaria digitata* was subjected to a two-stage fermentation process that involved hydrolysis followed by methanation. A comparison was made between single and two stage fermentation. It was found that two stage fermentation of *L. digitata* can be implemented if shorter retention times and higher organic loading rate are required. Average methane yields of 176 and 234 L/kg VS (two stage) and 221 L/kg VS (single stage) were obtained with higher methane compositions than that of the single stage process.

Hydrogen from surplus electricity can be reacted with CO₂ via the Sabatier process for production of methane. Ex-situ biological methanation was conducted at two thermophilic temperatures (55°C and 65°C) with methane compositions of 85–88% and volumetric productivities of 0.45 and 0.4L CH₄/L_{reactor} were observed at 55°C and 65°C after 24h respectively. *Methanothermobacter* species represent likely and resilient candidates for thermophilic biogas upgrading.

Thesis output

Chapters which have been published as papers or are currently under review in peer-reviewed journals:

Chapter 3:

Amita Jacob, Ao Xia, Jerry D. Murphy, 2015. A perspective on gaseous biofuel production from micro-algae generated from CO₂ from a coal-fired power plant. *Applied Energy* 148, 396-402. (published)

Chapter 4:

Amita Jacob, Ao Xia, Daryl Gunning, Gavin Burnell, Jerry D. Murphy, 2016. Seaweed Biofuel Derived from Integrated Multi-Trophic Aquaculture. *International Journal of environmental Science and Development* 7, 805. (published)

Chapter 5:

Amita Jacob Guneratnam, Ao Xia, Jerry D. Murphy, 2017. Comparative study of single and two stage mono-fermentation of brown seaweed *Laminaria digitata*. *Energy Conversion and Management* (published)

Chapter 6:

Amita Jacob Guneratnam, Eoin Ahern, Jamie A.FitzGerald, Stephen A.Jackson, Ao Xia, Alan D.W.Dobson, Jerry D. Murphy, 2017. Study of the performance of a thermophilic biological methanation system. *Bioresource Technology* 225, 308-315. (published)

Chapter 7: Conclusions and Recommendations

Appendix A Co-authored papers on third generation feedstock and two stage fermentation

Ao Xia, Amita Jacob, Christiane Herrmann, Muhammad Rizwan Tabassum, Jerry D. Murphy, 2015. Production of hydrogen, ethanol and volatile fatty acids from the seaweed carbohydrate mannitol. *Bioresource Technology* 193, 488-497.

Ao Xia, Amita Jacob, Christiane Herrmann, Jerry D. Murphy, 2016. Fermentative bio-hydrogen production from galactose. *Energy* 96, 346-354.

Ao Xia, Amita Jacob, Muhammad Rizwan Tabassum, Christiane Herrmann, Jerry D. Murphy, 2016. Production of hydrogen, ethanol and volatile fatty acids through co-fermentation of macro- and micro-algae. *Bioresource Technology* 205, 118-125.

Markus Voelklein, Amita Jacob, Richard O' Shea, Jerry D. Murphy, 2016. Assessment of increasing loading rate on two-stage digestion of food waste. *Bioresource Technology* 202, 172-180.

Contribution to the papers

Chapter 3: I was the first author of the paper and was responsible for the literature survey, calculations and methodology.

Chapter 4: I was the first author of the paper and was responsible for the literature survey, calculations and methodology.

Chapter 5: I was the first author of the paper and was responsible for the literature survey, experimental design, operation of the reactors, sample collection and analysis, evaluating the data.

Chapter 6: I was the first author of the paper and was responsible for the literature survey, experimental design, operation of the reactors, sample collection and analysis. Evaluation of the data with part of the work being shared by a master's student and a PhD student undertaking the microbial assessment.

Appendix A: Co-authored papers on third generation feedstock and two stage fermentation. I was the second author in the papers and was responsible for doing certain significant parts of the experiments, sample analysis, data collection and feeding of the reactors.

Nomenclature

BMP	biomethane potential assay
CH ₄	methane gas
CO ₂	carbon dioxide gas
CSTR	continuously stirred tank reactor
FOS/TAC ratio	(Flüchtige organische säuren /totales anorganisches carbonat) Ripley
H ₂	hydrogen gas
ha	hectare
HRT	hydraulic retention time
Mt	million tonnes
OLR	organic loading rate
TAN	total ammonical nitrogen
VFA	volatile fatty acids
VS	volatile solids
wwt	wet weight
P2G	power to gas approach
IMTA	integrated multi-trophic aquaculture
PBR	photo bio-reactor
NER	net energy ratio
EU	European Union
FAO	Food and Agriculture organisation
COD	chemical oxygen demand
SNSP	system non-synchronous penetration
HFM	Hollow Fibre Membrane Reactor
ORP	Oxidation Reduction Potential

1.Introduction

1.1 Introduction and background to thesis

The biofuel industry has been steadily growing in the provision of first and to lesser extent second generation feedstocks. Third generation biofuels such as those derived from algae (micro- and macro-) and power to gas systems are the subject of increasing research and from a commercial perspective may be found at demonstration scale or early commercialisation. It has been the goal of the European Union to move away from crop based biofuels that interfere with food production and cause inflation of food prices. Ligno-cellulosic wastes are energy intensive as they need appropriate pre-treatments to increase the rate of breakdown of the substrates for easy fermentation. Micro and macro algae have higher biomass productivities and are lignin free and can be produced by sequestering carbon emissions and fish farm derived waste streams. Ireland has a coastline of 7500 km which is a rich source of marine based kelp (brown seaweed) that can be used to produce hydrogen and methane via a two-stage process. However excessive removal of natural seaweed can affect the coastal environment. Another potential resource for the production of renewable gaseous fuel could be the issue of curtailment of wind energy generated in Ireland. Curtailment can be minimised by adopting a power to gas approach (P2G), wherein the surplus wind electricity can be converted to hydrogen (via electrolysis) and then transformed to methane using CO₂ via the Sabatier reaction through biological (or catalytical) methanation. This is considered as a third-generation biofuel from non-biological source as the hydrogen gas is derived from surplus wind electricity as opposed to biomass.

Process optimisation and factors affecting process stability need to be continuously studied and researched at laboratory and pilot scale to test the techno-economic viability of these technologies before complete implementation. This thesis mainly investigates the production of third generation gaseous biofuels from algal and non-biological sources.

1.2 Thesis aims and objectives

The aims and objectives of the thesis were as follows:

Micro-algal biogas associated with carbon capture at a coal fired power plant

- To study the process of carbon capture via micro-algae from coal power plant emissions and calculate the quantities of algal biomass that can be produced using open and closed cultivation systems.
- To estimate the footprint needed to cultivate micro-algae along with a brief view on the parasitic energy demand that will determine the method of cultivation and the viability of this technology.
- To assess the production of gaseous biofuels (hydrogen and methane) from micro-algae subjected to a three-stage sequential fermentation process of dark, photo fermentation and anaerobic digestion.

Macro-algal biogas sourced from seaweed in an integrated multitrophic aquaculture system

- To investigate the importance of aquaculture as a source of seaweed production for food, hydrocolloids and biogas production.
- To highlight the interdependence of the fish industry and seaweed production to mitigate the environmental disturbance caused by fish farms.
- To calculate the production of seaweed (*Saccharina latissima*) derived from waste streams of fish farms using integrated multi-trophic aquaculture (IMTA) whilst studying a simplified model to provide 1.25 % of energy in transport (as bio-methane) from coastal digesters digesting seaweed derived from IMTA

Single and Two stage production of biogas from seaweed

- To examine the effect of single versus two stage digestion of the seaweed *Laminaria digitata* on its process parameters such as hydraulic retention time and organic loading rate.
- To identify the merits of the two digestion systems based on their overall energy yield, methane composition and yields.
- To estimate the hydrogen yield and VFA composition of the hydrolysis effluent for a comparison with those found in literature for seaweed and its sugars.

Biological methanation in a power to gas system

- To study the process of biological methanation using H₂ and CO₂ as input gaseous substrates at two different thermophilic temperatures whilst examining the effects of time and temperature on the rate of reaction.
- To investigate its methane composition, volumetric productivity and microbial diversity responsible for methanation.

1.3 Thesis outline and link between chapters

This thesis is a compilation of 8 chapters starting from introduction to conclusions and recommendations. Chapter 2 examines the state of the art in third generation gaseous biofuels. Chapters 3 to 7 outline the research undertaken both desktop and laboratory based. Chapters 3 to 7 are either published or submitted for publication to peer review journal press. A short summary of these chapters (2-7) is given below.

Chapter 2: Literature review of gaseous biofuels from micro, macro algae and biological methanation

This chapter focuses on the work that has been done on micro algae; their production (using raceway ponds, tubular and flat plate photo bioreactors) from carbon capture of emissions from industry and their subsequent utilisation to produce hydrogen and methane via dark, photo fermentation and anaerobic digestion. Chapter 2 also gives a perspective on seaweed/macro-algae and its production from fish farms through IMTA. A perspective is also given on how the use of seaweed could potentially be a little controversial as it is used as food in most Asian countries and fetches a higher price to produce chemical commodities such as hydrocolloids that are used in various industrial applications. A literature search on two stage digestion of *Laminaria digitata* shows that there is a scarcity of studies on continuous two stage digestion of this seaweed. The chapter ends with a literature review on biological methanation using biogas, hydrogen, carbon dioxide as various input gases (both in-situ and ex-situ). Biogas is used where the reaction is carried out in-situ and the process can also be termed as upgrading. The experiment done in this thesis was ex- situ with the use of H₂ and CO₂ as input gases.

Chapter 3: A perspective on gaseous biofuel production from micro-algal generated from CO₂ from a coal-fired power plant

Chapter 3 is a desktop study on the potential of carbon capture using emissions from coal power plants. It discusses the characteristics of exhaust from such plants, the different methods of cultivating micro-algae with their merits and demerits. It was found that tubular photo bioreactors have the highest biomass productivity and have a smaller footprint than raceway ponds and flat plate reactors; however tubular photo bioreactors are expensive to operate and are energy intensive, and it was concluded that any benefits arising from the production of gaseous biofuel from the micro-algae produced using such a system.

Chapter 4: Seaweed Biofuel derived from Integrated Multi-trophic Aquaculture

Chapter 4 is also a desktop study and explores the other form of algae i.e. seaweed (macro-algae). It sheds light on global production of seaweed (both natural and aquaculture derived) and the various industrial applications of seaweed and its consumption as food. Seaweed production can be tied with the fish industry as fish protein is becoming an increasingly important source of protein globally. The negative effects of such large-scale fish farms can be reduced by sequestering the waste from such farms to grow seaweed and use it for biogas production.

Chapter 5: Comparative study of single and two stage mono-fermentation of brown seaweed *Laminaria digitata*

Chapter 5 is laboratory work where 4 reactors were operated at mesophilic temperature for mono-fermentation of *L. digitata*. Two stage digestion comprising of hydrolysis (production of hydrogen and VFAs) and methanation (the VFAs produced in the hydrolysis reactor were consumed to form methane) were employed along with a single stage digestion process (digesting seaweed directly). Factors such as hydraulic retention time, organic loading rate pH and the profile of volatile fatty acids were monitored and studied to see its effect on methane yield and composition.

Chapter 6: Study of the performance of a thermophilic biological methanation system

Chapter 6 is laboratory work where 3 reactors were set up with mixed culture as inoculum to produce methane using H₂ and CO₂ as input substrate gases. The reactor contents were replenished with 25 ml of nutrient medium (with 25 ml of digestate withdrawn daily) to provide essential vitamins and minerals for the growth and sustenance of the microbes. The reactors were run at thermophilic temperatures of 55 and 65°C. The variability in methane composition and volumetric productivity due to temperature and retention times were

monitored and studied. Microbial analysis was also done to investigate the species responsible for biological methanation at thermophilic temperatures

Chapter 7: Conclusions and Recommendations based on the work done in the thesis

Appendix A: Co-authored papers on third generation feedstock and two stage fermentation.

This gives a summary of the papers where certain significant parts of the experiment were conducted by me to add to this thesis. These papers fall well in line with the theme of my thesis that also deals with algal (micro and macro) biomass. A detailed study on the effect of increasing organic loading on two stage digestion of food waste was done. This helped to the understanding of running the same process for the brown seaweed *Laminaria digitata*.

2. Literature review of gaseous biofuels from micro, macro algae and biological methanation

2.1 Micro and macro algal biomass

Micro-algae: Biological fixation of CO₂ can be achieved using micro algae that possess high biomass productivity when compared to terrestrial crops. These tiny mostly autotrophic organisms have very high rates of multiplication and can have biofuel yields of nearly 10-100 times those of terrestrial crops because of their high productivities [1]. Microalgae can be cultivated in seawater or waste water, they can be suitably used to sequester industrial CO₂. Microalgae can exist as bacteria (cyanobacteria), diatoms or unicellular plants such as chlorophyta; some species can contain more than 70% lipids, and some could be high in proteins such as *spirulina*. Micro-algal growth can be affected by rates of CO₂ absorption, intensity and duration of illumination, availability of nutrients and method of cultivation such as: open systems (raceways ponds); or closed systems (tubular, parallel, bubble column, airlift, biofilm or flat plate reactors) [2]. By varying the above factors different species of micro-algal strains can be grown to produce biodiesel or biogas. To reduce operating costs and to increase CO₂ absorption, an indirect process can also be used whereby CO₂ is captured as bicarbonate and used in a liquid medium to cultivate micro-algae [3].

Cultivation of micro-algae using carbon emissions from various kinds of industries such as coal power plants, cement manufacturing plants have been attempted. Coal power plants have been studied extensively as it a major source of electricity production with high carbon footprint. Several lab scale and pilot plant studies have been conducted to test the viability of flue gas as a source of carbon to produce micro-algae. Satisfactory growth of micro-algae was observed under varying conditions of temperature, micro-algal strain, CO₂ percentage and NO_x/SO_x concentrations in the flue gas. Maximum biomass productivity (1000 mg/L/d) was obtained for the *Chlorella* sp. at 25°C and 15 % of CO₂ at a CO₂ consumption rate of 1880 mg/L/d [4]. To improve gas transfer in the micro-algal medium, different reactors were tested such bubble column, airlift, tubular and flat plate reactors and open ponds. However

hollow fibre membrane reactors were the most efficient for gas mass transfer [5]. Despite technological advances, cost of cultivation of microalgae is still an issue for its use as a biofuel feedstock [6].

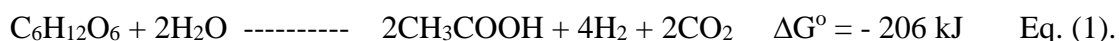
Macro-algae: Also, known as seaweed is found naturally on coastlines or in eutrophic waters. Its growth is accelerated at high nitrogen content in the water [7]. Seaweed is traditionally eaten as food in some Asian countries (Kombu, Nori). Seaweed contains typically 20% dry matter and is lignin free and is easy to degrade when compared to terrestrial counterparts [8]. Seasonal variation of the carbohydrate content in seaweed can be an impediment to its successful implementation in the use of biogas production. The main polysaccharide molecules found in seaweed are alginate, mannitol, laminarin and fucoidan. Several pigments such as polyphenols are also present in certain seaweeds. As seaweeds are mostly grown in seawaters, they are an excellent source of minerals. Some of the most common metals/minerals found in the intracellular parts of the brown seaweed *ascophyllum nodosum* are zinc and manganese (60 and 38 mg/kg dry weight) while traces of cobalt, chromium, copper and nickel were found as well [9]. Brown seaweeds contain more of the above-mentioned metals than red and green seaweeds. Because of the high metal sequestration capacity of seaweeds, it can effectively be used as a tool to clean waste waters that are released by fish farms. The growth of seaweeds in such highly rich nitrogenous waters can give high yields of biomass that can be used to produce biogas. Integrated multi-trophic aquaculture can be implemented to clean up fish farms that excrete nitrogenous substances into the water; seaweeds sequester inorganic waste and shellfish sequester organic waste [10]. Studies done on a Salmon farm near Chile showed biomass productivity of 53 g/m² /d for *Gracilaria chilensis* with a nitrogen removal capacity of 9.3 g/m for long line cultivation [11]. It has also been found that nitrogen sequestration by *P. palmata* and *S. latissima* can

remove up to 12% and 5% of the nitrogen released from the growth period of 500 tonnes of salmon in the sea over 2 years [12].

2.2 Photofermentation, Dark fermentation/hydrolysis and anaerobic digestion

Hydrogen gas (H₂) is considered to be a clean burning fuel; several thermochemical methods reliant on the petro-chemical and coal industries are still used for the mass industrial production of hydrogen; these methods are expensive, energy intensive and do not constitute renewable hydrogen. Biohydrogen produced from biological processes are considered to be less expensive however they are yet to be proven viable for industrial application due to the low yields obtained in processes such as dark/photo fermentation [13]. Dark fermentation is the fermentative breakdown of carbohydrate molecules (higher and lower polysaccharides) resulting in the production of hydrogen and volatile fatty acids (VFAs). For higher yields and productivities of H₂ the VFAs can then be subjected to photo fermentation.

Dark fermentation: also known as hydrolysis is the breakdown of all complex carbohydrates in the reactor under anaerobic conditions; mediated by a wide variety of bacteria, such as the spore forming *Clostridium* species, facultative *Enterobacter sp*, *Bacillus sp*. This process is enzymatically catalysed by hydrogenases that mainly act on monosaccharides as their carbon source (glucose), that are obtained by hydrolysis of polysaccharides. Dark fermentation results in the conversion of glucose to H₂, acetic acid and CO₂ as given in Eq. (1). This process has a high negative free energy and as a result it is a highly spontaneous reaction; a maximum of 4 mol of H₂ (theoretically) can be obtained per mole glucose if acetic acid is the only VFA product[14].



Microbial growth and maintenance also need energy hence lower yields than theoretical are obtained. The butyric acid pathway leads to 2 mol of H₂ per mole of glucose. The optimal pH for dark fermentation is found to be between 5-6.5. A pH of lower than 5 will result in lactic acid and ethanol formation that do not contribute to H₂ production. Co-formation of acetic and butyric acids results in 2.5 mol H₂ per mole glucose.

Thermophilic temperatures result in higher hydrogen yields (40-65°C) as they prevent the growth of hydrogen consuming bacteria and promote the rate of the reaction by increasing metabolic activity. Certain nutrients help in the growth of the bacteria such as nitrogen phosphorous, iron and sulfur[15].

Photofermentation: Photosynthetic non-sulfur (PNS) bacteria can convert VFAs to H₂ and CO₂ under anaerobic conditions; they could also use carbon sources like glucose, sucrose and succinate rather than VFAs [16]. *Rhodobacter sphaeroides* O.U001, *Rhodobacter capsulatus*, *R. sphaeroides*-RV, *Rhodobacter sulfidophilus*, *Rhodopseudomonas palustris* and *Rhodospirillum rubrum* are some of the commonly used (PNS) bacteria [16]. Along with hydrogenase, nitrogenase is the main enzyme catalysing this reaction with a positive free energy (Eq 2). As such the reaction does not proceed spontaneously and needs an external energy source in the form of light, with appropriate wavelengths and intensities of 400-1000nm and 6-10 klux. The ideal operating conditions were obtained at a pH of 6.8-7.5 and a temperature in the range of 31-36°C [17]. As Fe and Mo are the main co-factors present in nitrogenase, addition of such trace elements can enhance H₂ production[13, 16].



Anaerobic Digestion/Methanation: the production of methane from VFAs can be achieved through methanation under anaerobic conditions; methanogens such as hydrogenotrophic and acetoclastic archaea produce methane from hydrogen, carbon dioxide and acetic acid respectively [18]. The process functions at its optimum level at a pH between 7-7.5. The hydraulic and solid retention time (HRT and SRT) are key process parameters and should be sufficiently high enough to allow the active populations of microbes to remain in the reactor, especially methanogens since they have a long doubling time [19]. Mesophilic and thermophilic temperatures have been utilised for increasing the performance of the reactor. However at high temperatures ammonia inhibition can take place as proteins breakdown faster releasing free NH_3 into the liquid state in the reactor that cause toxicity in the cell structure of methanogens. A very high concentration of VFAs (>1000 mg/L) can cause the pH to reduce and lower methane yields eventually causing reactor failure [20].

2.3 Gaseous biofuels production from micro and macro algae

Gaseous biofuels from micro-algae: Hydrogen and methane have been derived from micro-algae through sequential fermentation as shown in Table 2.1. Most of these studies have been done at the lab scale (batch) with very few studies done for continuous digestion process. Carbohydrate rich micro-algae are suitable for the production of hydrogen and methane. Species such as *Chlorella pyrenoidosa* and *Nannochloropsis Oceanica* are rich in high molecular weight carbohydrates such as xylan and glucans that necessitate pretreatment of micro-algae (steam pretreatment, microwave heating, methods coupled with dilute acid treatment) to obtain low molecular weight sugars (saccharides) such as xylose and glucose for efficient hydrolysis. Micro-algae rich in proteins such as spirulina may not be suitable for hydrogen or methane production as excessive protein breakdown can cause unionised ammonia inhibition during fermentation leading to process failure and low yields of hydrogen and methane.

Table 2. 1: Gaseous biofuel production from micro-algae

Substrate	H ₂ yield (ml H ₂ /g VS)	CH ₄ yield (ml CH ₄ /g VS)	Mode of operation
<i>Chlorella pyrenoidosa</i> ¹	198.3	186.2	Batch
<i>Nannochloropsis</i> <i>Oceanica</i> ¹	183.9	161.3	Batch
<i>Arthrospira platensis</i> ²	354.7	-	Batch
<i>Arthrospira maxima</i> ³	82.8	115.3	Batch
<i>Chlorella pyrenoidosa</i> ⁴	ca.20	-	Batch
<i>Nannochloropsis</i> ⁴ <i>Oceanica</i>	ca.20	-	Batch
<i>Arthrospira platensis</i> ⁵	-	330.2	Continuous

1: subjected to sequential dark fermentation, photo-fermentation and anaerobic digestion [1], [21].

2: subjected to sequential dark fermentation and photo-fermentation [22].

3: subjected to sequential dark fermentation and anaerobic digestion [15].

4: subjected only to dark fermentation [23]

5. subjected only to anaerobic digestion/methanation [24]

Gaseous biofuels from macro-algae: Macro-algae has been used to produce liquid biofuels; however downstream processing of fuels such as ethanol and biodiesel are very energy intensive and hence gaseous biofuel production via anaerobic fermentation is deemed more feasible as macro-algae are not high in lipids and it is more prudent to produce hydrogen and methane than biodiesel. Macro-algae contain carbohydrates and essential minerals such as cobalt, nickel and zinc that may aid in fermentation as these minerals are considered to help microbial growth and maintenance as they form co-factors for certain enzymes that are

produced by microbes for hydrolysis and methanation. Liquid phase fermentation is more feasible as seaweed contains close to 75-90% water that can increase the cost of drying and cause problems associated with storage and transportation. Coastal digesters can be used to produce biogas from seaweed to alleviate these problems. Plenty of batch scale studies have been done on macro-algae to produce hydrogen and methane. However, very few studies have focussed on continuous digestion. Table 2.2 gives the gaseous biofuel yields of some types of seaweed that have been studied for their hydrogen and methane production.

Table 2. 2: Gaseous biofuel production from macro-algae

Substrate	H ₂ yield (ml H ₂ /g VS)	CH ₄ yield (ml CH ₄ /g VS)	Mode of operation	Reference
<i>Ulva lactuca</i> ¹	-	271	Batch	[25]
<i>Laminaria digitata</i> ²		238	Batch	[26]
<i>Saccharina latissima</i> ³		340	Batch	[27]
<i>Ascophyllum nodosum</i> ⁴		110	Batch	[28]
		47	Batch	[29]
<i>Gracilaria</i> ⁵ <i>vermiculophylla</i>		295	Batch	[30]
<i>Laminaria digitata</i> ⁶	ca.80ml	-	Batch	[23]
<i>Saccharina latissima</i> ⁷	ca.35ml	-	Batch	[23]
<i>Laminaria japonica</i> ⁸	113 ^a	250-300 ^b	Batch for H ₂ Continuous for CH ₄	[31]

1-5: subjected only to anaerobic digestion(methanation)

6,7: subjected only to dark fermentation

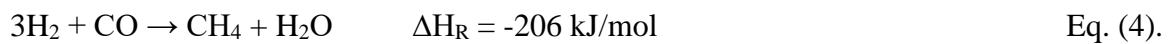
8 : subjected to sequential dark fermentation and anaerobic digestion(methanation)

a : mL H₂/g dry cell weight

b : mL CH₄/g COD

2.4 Biological methanation

Methanation of hydrogen and carbon dioxide is a process that can be mediated by chemical or biological catalysts such as methanogenic archaea. Methane can be produced by reacting hydrogen with either carbon monoxide or carbon dioxide. described by Eq. (3). (Sabatier Equation) or by Eq. (4).



Biological methanation carried out by methanogenic archaea is generally the final step in the anaerobic digestion process utilising CO_2 , H_2 , acetate, formate or other alcohols as substrate to form methane. Some, such as those belonging to the order *methanosaeta*, may only utilise acetate, while other orders such as *methanosarcina* are more flexible and can utilise either acetate or $\text{H}_2 + \text{CO}_2$. Methanogens generally grow at 35-70°C[32]. Biological methanation may be carried out at industrial scales, typically in conjunction with a conventional biogas plant; in such a case hydrogen is injected into an anaerobic digester digesting grass, maize or food waste. Such a process can be deemed as in-situ biological methanation whereas ex-situ methanation can be carried out in a separate stainless steel vessel with the injection of hydrogen and carbon dioxide that are consumed by methanogenic culture. Various reactor designs have been studied particularly to improve the gas transfer rate of hydrogen into the liquid state as it is a sparingly soluble gas in water when compared to carbon dioxide. Apart from reactor design, a number of other process variables that can affect the performance of the reactor are temperature, mechanical mixing rates, gas flow rates and the specific strains of methanogens utilised. A review of the various designs available in the literature is presented in Table 2.3.

Table 2. 3: Existing reactor designs and performance data

Reactor	Temp (°C)	Inoculum	Influent gas	Operation mode	Working volume (L)	Max methane concentration (%)	Reference
CSTR	55	Anaerobic digestate	Biogas + H ₂	Continuous	0.6	95.4	[33]
Trickle bed with packing	37	Anaerobic digestate	H ₂ + CO ₂	Batch	88	96	[34]
Up-flow bed	35	Anaerobic digestate	H ₂ + CO ₂	Continuous	7.8	-	[35]
HFM	37	Anaerobic digestate	H ₂ + CO ₂	Continuous	0.195	85	[36]
CSTR	37	Anaerobic digestate	H ₂ + CO ₂	Continuous	100	92	[37]
CSTR	60	Pure culture	Biogas+ H ₂ H ₂ + CO ₂	Continuous	3	-	[38]
CSTR	65	Pure culture	Biogas + H ₂	Continuous	10	85	[32]

CSTR: Continuous Stirred Tank Reactor; HFM: Hollow Fibre Membrane Reactor

Biogas plants that aim for absolute natural gas quality methane would try to operate the reactor at maximum possible efficiency to obtain methane concentrations as high as 95-98%. Solubility of the gas can be increased by providing a larger surface area such as trickle bed and hollow fibre membrane reactors with packing (see Table 2.3) or by increasing the retention time of the gas in the reactor by designing tall reactors. Mechanical mixing via stirring in a continuously stirred tank reactor (CSTR) is probably the simplest method of assisting H₂ to go into solution. Another alternative to mechanical mixing is micro-sparging. In this case, the gas is released into the liquid via micro-porous material, such as a hollow fibre membrane (HFM) [35, 36] creating small hydrogen bubbles with high partial pressure

and a high ratio of surface area to volume. With the help of this literature review, the desktop work and experiments have been designed to fill in the literature gap as well as shed light on third generation gaseous biofuels from algae and non- biological sources.

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3. A perspective on gaseous biofuel production from micro-algae generated from CO₂ from a coal-fired power plant

A perspective on gaseous biofuel production from micro-algae generated from CO₂ from a coal-fired power plant

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Abstract

There are significant resources of coal on the planet. It is likely that a lot of this coal will be combusted. A 1 GWe coal power plant operating at 35% electrical efficiency and a capacity factor of 75% produces 6.72 million tonnes of CO₂ per annum. A closed cultivation system with a carbon capture efficiency of 80 % allows production of 2.69Mt of micro-algal (volatile solids), in a foot print of 19,200 ha for a tubular photo-bioreactor (PBR) and 34,000 ha for a Flat Plate PBR. An open system (raceway pond) at a carbon capture efficiency of 50 % produces 1.68Mt of micro-algal (volatile solids) and requires a footprint of 52,303 ha. Employing a three-stage sequential process (combining dark fermentation, photo fermentation and anaerobic digestion) to produce bio-hydrogen and bio-methane from the micro-algae could potentially generate 35% of the primary energy in the coal in the form of renewable gaseous fuel if a closed system of cultivation is used. This is sufficient to fuel 600,000 cars per annum. In the cultivation of micro-algae, pumping and circulation is a considerable parasitic energy demand. The ratio of energy output (gaseous biofuel) to energy input (pumping and circulation) is less than 1 for all the three cultivation systems assessed, ranging from 0.71 for raceway ponds to 0.05 for a tubular PBR. If coal powered electricity is the source of this parasitic energy, then a tubular PBR system produces more CO₂ than the CO₂ captured by the micro-algae.

Keywords: coal; gaseous biofuel; micro-algae; CO₂ fixation; bio-hydrogen; bio-methane.

3.1 Introduction

3.1.1 Coal: a cheap and plentiful fossil fuel

Coal contributed 29.9% of the world's primary energy needs and 41% of global electricity production in 2013 and remains the predominant source of electricity production in countries like India and China [1, 2]. Coal is a relatively cheap fossil fuel; widely distributed across the world (Figure 3.1); proven coal reserves in 2013 were sufficient to meet 113 years of global production. This is the highest Reserves/Production (R/P) ratio for any fossil fuel; natural gas and oil have R/P ratios of 55.1 and 53.3 respectively [3]. An estimate from the World Bank suggests that over 1.2 billion people still remain without access to commercial energy supplies; this is particularly the case for electricity [1]. Coal, being the cheapest and most abundant fossil fuel resource in non-OECD nations, will more than likely be used as a primary source of power generation. Developed OECD nations such as the US, Russia and Australia also have large indigenous coal reserves that will most likely be utilised to produce electricity at the cheapest cost. Coal combustion contributed 40% to global CO₂ emissions with a share of 28% from coal-fired power plants in 2012 [4]. CO₂ emissions from coal combustion increased by 4.9% in 2011 compared to 2010 [5].

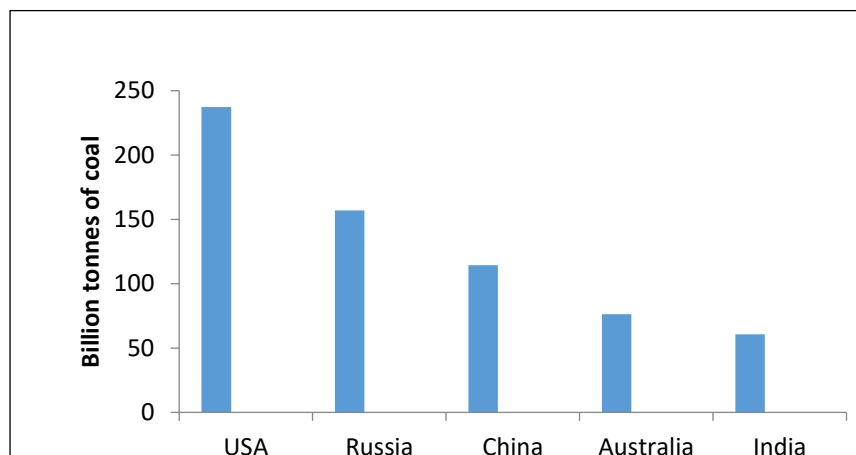


Figure 3. 1: Proven coal reserves in the top five coal producing countries [3].

3.1.2 Capture of CO₂

Absorption of CO₂ is effected using various chemical agents such as Monoethanolamine (MEA), solid adsorbents like activated carbon, or zeolite 5A [6-8]. Membranes and cryogenic fractionation have also been employed for the removal of CO₂ [7]. The chemical methods of CO₂ separation are highly energy intensive and expensive [6, 9]. Conventional carbon capture technologies (largely using chemical methods) have a capture efficiency of 85-95% [10]. It has been reported that 3.7 GJ of energy/tonne of CO₂ absorbed is required during the regeneration of MEA, which corresponds to around 370 kg of extra CO₂ (per t CO₂ absorbed) emitted if this energy input comes from a fossil fuel such as coal [7].

3.1.3 Cultivation of micro-algae using flue gases from industries as a source of CO₂

3.1.3.1 Suitability of flue gases to provide CO₂ to micro-algae

Flue gases from coal power plants can be a potential CO₂ source to produce micro-algal biomass [9, 11]. Micro-algae can utilize CO₂ with the help of solar energy, ten times more efficiently than terrestrial plants [12, 13]. Micro-algae can be grown in saline conditions or wastewater throughout the year [14]. Flue gases are generally dominated by N₂ (72-74%), CO₂ (4.8-26.9%), H₂O (9-13.8%) and O₂ (0.7-15%). However, they also contain smaller quantities of NO (59-1500 mg/Nm³), NO₂ (2-75mg/Nm³), SO₂ (20-1400 mg/Nm³), SO₃ (0-32 mg/Nm³), CO (100-11250 mg/Nm³), particulate matter (2000-15000 mg/Nm³) and heavy metals (2.2 mg/Nm³) [9, 15]. Typically, flue gases are treated for the removal of particulate matter, heavy metals and NO_x and SO_x to comply with the regulations on effluent discharge and air quality set by the Clean Air Act that is monitored by the Environmental protection agency.

3.1.3.2 Factors effecting growth of micro-algae

CO₂ levels of 10-16% (v/v) can be obtained from pulverised coal-fired power plants with 100-1000ppmv of NO and 100-2000ppmv of SO₂ [7]. Studies conducted on the flue gas composition of two coal-fired power plants in Australia showed NO_x and SO_x levels of up to 524ppmv and 258.9ppmv (wet basis) [16]. Several species of micro-algae can easily uptake CO₂ up to a level of 18-20%, with most strains of micro-algae exhibiting favourable rates of CO₂ fixation [6-8]. Typically, levels of NO_x and SO_x below 100ppm and 50 ppm have no effect on micro-algal growth, however raw flue gas contains higher levels, hence flue gas may need to be pre-treated [7, 9, 17]. Apart from carbon, nitrogen and phosphorus also play an important role in cell development and growth. Nitrogen is important to produce proteins, nucleic acids whereas phosphorous helps in growth and maintenance of optimum pH.

Micro-algal strains such as *Chlorella* Sp. T-1 can be cultivated by direct injection of flue gas with little hindrance from the high levels of NO_x and SO_x. Optimum growth was observed at 10% CO₂ concentration. However, no inhibition to growth was found at 50%, 80% or 100% CO₂ [17]. The temperature of flue gases from industries can be in the range of (430-950K). However, a temperature range of 20°C to 35 is considered favourable for the growth of micro-algae [17, 18].

3.1.3.3 Micro-algae production systems

Micro-algae can be grown in two ways: closed systems (photo-bioreactors) and open systems (raceway ponds) [19-21]. Open systems are cheaper, easy to clean and require lower capital investments; however, they are not a technically sound choice since rates of water evaporation and CO₂ loss are very high [21, 22]. Operational parameters, such as

temperature, light utilization, mixing and process control, are very difficult to maintain in such systems.

Photo-bioreactors (closed systems) have higher productivities than open systems with better process control; they occupy less space and are technically more advanced than open systems. They have higher costs of installation and operation [19, 21-23].

The CO₂ fixation efficiency of *Chlorella vulgaris* was reported to be 74% and could vary for different strains of micro-algae [8]. The remainder of the carbon may be found in the form of bicarbonates in the culture medium, with a smaller fraction potentially leaving the system as carbon dioxide (which has lower solubility at higher temperatures). Carbon capture efficiencies could be as high as 90% for closed systems such as photo-bioreactors [24], with some reports suggesting a range between 45%-70% [25]. Raceway ponds have lower efficiencies (25% -50%) and are prone to contamination and high rates of water losses [25].

3.1.4 Gaseous biofuel production from micro-algae

3.1.4.1 Bio-hydrogen from dark and photo-fermentation

The process of anaerobic digestion can be sub-divided into phases and optimised for each consortium of microbes. In a two-stage system, the first stage can be used to produce volatile fatty acids (VFA) which can then be fed to a second phase methane reactor. The first phase known as dark anaerobic fermentation requires a low pH (5 - 6), a high organic loading rate (ca. 20 kg COD/m³/d), and a short retention time (ca. 2 days) [26]. Dark anaerobic fermentation produces a biogas rich in hydrogen and carbon dioxide [27-29].

Small chain fatty acids in the effluent of dark fermentation can be utilised by photosynthetic non-sulphur (PNS) microbes to produce hydrogen under anoxic conditions in the presence of light thereby increasing the hydrogen yield. This process is known as photo-fermentation. A pH of 6.8-7.5, a temperature range of 31-36°C and a wavelength of 400-1000nm have been reported to be the optimum operating parameters for photo-fermentation [27]. The effluent

from dark fermentation needs to be treated if it contains high concentrations of ammonium ion (NH_4^+) (exceeding 40ppm) which can inhibit activity of the nitrogenase enzyme which mediates hydrogen generation. Fe and Mo (co-factors of nitrogenase) are also required for the optimum performance of this enzyme [27, 30, 31].

Theoretically if complete degradation takes place then 1 mole of glucose can liberate 12 moles of H_2 by a sequential dark and photo-fermentation process when acetic acid is the sole VFA obtained [27, 29]. However, such ideal yields are not obtained and a yield of at least 8mol of H_2 /mol of glucose is expected to make the process economically practical [27, 32]. A high experimental yield was obtained by a sequential dark and photo-fermentation of a medium containing 10g/L of sweet potato starch (7.2mol H_2 /mol of glucose) [33].

3.1.4.2 Bio-methane

Bio-methane production has been studied for algal residue from micro-algae derived bio-diesel, as well as micro-algae as a raw material [11, 34, 35]. Experimental yields ranging from 200- 450 L CH_4 / kg VS have been reported in the literature for continuous as well as batch reactors [11-13, 34]. The initial focus of biofuel studies from micro-algae was on biodiesel; however, drying of the micro-algae prior to the bio-esterification process and the subsequent high cost of lipid extraction is a major hindrance to the energy balance [20, 23, 36]. There is limited literature examining a three-stage continuous process for the combined production of hydrogen and methane via dark fermentation, photo-fermentation and anaerobic digestion. One study reported values of 82.8 ml H_2 /g VS and 115 ml CH_4 /g VS on dark fermentation followed by anaerobic digestion for *Arthrospira maxima* [37]. Higher yields of up to 198.3 ml H_2 /g VS and 186.2 ml CH_4 /g VS have been obtained for dark fermentation, photo-fermentation and anaerobic digestion of *Chlorella pyrenoidosa*.

3.1.4.3 Bio-hydrogen and bio-methane from micro-algae

Several species such as *A. maxima*, *C. vulgaris*, and *C. pyrenoidosa* have been studied for bio-methane and bio-hydrogen production. Pre-treatments such as steam heating with dilute acid and ultra-sonication and enzyme treatments have been used to increase the yield of the gaseous fuels produced [12, 13, 35]. Researchers at the Zhejiang University conducted a three stage sequential dark fermentation, photo-fermentation and anaerobic digestion of pre-treated *C. pyrenoidosa* and effected an experimental yield of 198.3 ml H₂/g VS and 186.2 ml CH₄/g VS [12] . Table 3.1 gives the energy yields of micro-algae via combined dark fermentation, photo-fermentation and anaerobic digestion.

Table 3. 1: Energy yields obtained from sequential fermentation and digestion of micro-algae

Substrate	H ₂ yield (ml H ₂ /g VS)	Energy yield (kJ/g VS) ⁴	CH ₄ yield (ml CH ₄ /g VS)	Energy yield (kJ/g VS) ⁴	Total energy yield (kJ/g VS)	Reference
<i>Chlorella pyrenoidosa</i> ¹	198.3	2.1	186.2	6.7	8.8	[12]
<i>Nannochloropsis Oceanica</i> ¹	183.9	1.98	161.3	5.77	7.75	[13]
<i>Arthrospira platensis</i> ²	354.7	3.8	-	-	3.8	[38]
<i>Arthrospira maxima</i> ³	82.8	0.89	115.3	4.12	5	[37]

1: subjected to sequential dark fermentation, photo-fermentation and anaerobic digestion.

2: subjected to sequential dark fermentation and photo-fermentation.

3: subjected to sequential dark fermentation and anaerobic digestion.

4: calculated using lower heating values of H₂ (10.78 MJ/m³) and CH₄ (35.8MJ/m³)

3.1.5 Energy consumption and carbon emissions.

The proposed system captures carbon dioxide emissions through production of micro-algae but as these micro-algae are used to produce biofuels that will be combusted, the carbon dioxide captured will eventually be re-released to the atmosphere. Total energy from the proposed system has been increased as a gaseous fuel is now produced from the original coal. However energy is required to produce micro-algae and hence indirect emissions have to be taken into consideration. It has been observed that the energy used to pump the micro-algal culture contributes ca. 79 % of the total energy used in raceway ponds and 92 % of the total energy used in tubular photobioreactors [21]. This paper does not purport to undertake a full energy audit but will examine the parasitic energy demand required for pumping and circulating the culture medium and express this as a ratio of the energy output in the gaseous fuel.

3.1.6 Technology readiness level of biological carbon capture

Efforts are being made for large scale implementation of bio-sequestration of CO₂ using micro-algae on an industrial level. Several companies have implemented this method of biological carbon sequestration and recycle. *Seambiotic, Ashkelon, Israel* use flue gases from the Israel Electric Company to grow micro-algae for numerous applications in the field of fine chemical, pharmaceuticals and biodiesel. Other companies operating in this sphere include *A₂BE Carbon Capture*, Boulder, and *Solix Biofuels*, Fort Collins in Colorado [6]. Most recently *AlGAE.TEC*, a company founded in Australia have signed a deal with Macquarie Generation (owned by the New South Wales government) to build a large algae facility which will sequester carbon emissions from the 2.64GWe coal-fired power plant at Bayswater in Hunter Valley, New South Wales. They initially plan to capture 270,000 tonnes of CO₂ and increase this to 1.3 million tonnes in the next few years. This coal power plant

uses about 7.5 million tonnes of coal with an annual CO₂ emission of about 19 million tonnes [39, 40].

However, the sequential process of dark fermentation, photo-fermentation and anaerobic digestion has not been tested in a commercial setting. Several lab scale studies have been done to establish the proof of concept and to improve the efficiency of the process [12, 13].

3.1.7 Aims and Objectives

The aim of this chapter is to present a perspective on an innovative biological capture system of CO₂ from a coal-fired power plant complete with generation of renewable algal biofuel. The investigation assessed the scale, the energy return and the CO₂ efficiency of the process.

The objectives of this chapter are to:

- Assess the potential yield of micro-algae through use of CO₂ emissions from a 1GWe coal- fired power plant.
- Assess the footprint required for micro-algal cultivation.
- Calculate the potential production of renewable gaseous fuel in the form of bio-hydrogen and bio-methane from micro-algae through a three-stage sequential process combining dark fermentation, photo fermentation and anaerobic digestion.
- Provide a perspective on the parasitic energy demand of the three different cultivation systems.

3.2 Analysis of carbon capture and micro-algal gaseous biofuel system

3.2.1 Production of micro-algae using CO₂ emissions from a coal-fired power plant.

Carbon emissions from a 1GWe coal-fired power plant can be captured to grow micro-algae in closed systems such as tubular photo-bioreactors. Figure 3.2 broadly illustrates the idea proposed. Typically, to comply with discharge regulations for gaseous effluents, a pre-treatment process consisting of De-NO_x and De-SO_x is used; installation of such units is

prudent if the strain of the micro-algal species is sensitive to high levels of these compounds. Levels of NO_x and SO_x should be brought down to 100 and 50 ppm respectively [7, 9, 17]. Excess heat from the flue gas can be used to dry the micro-algae for down-stream processing or for use as a source of heat treatment prior to digestion by using a heat exchanger.

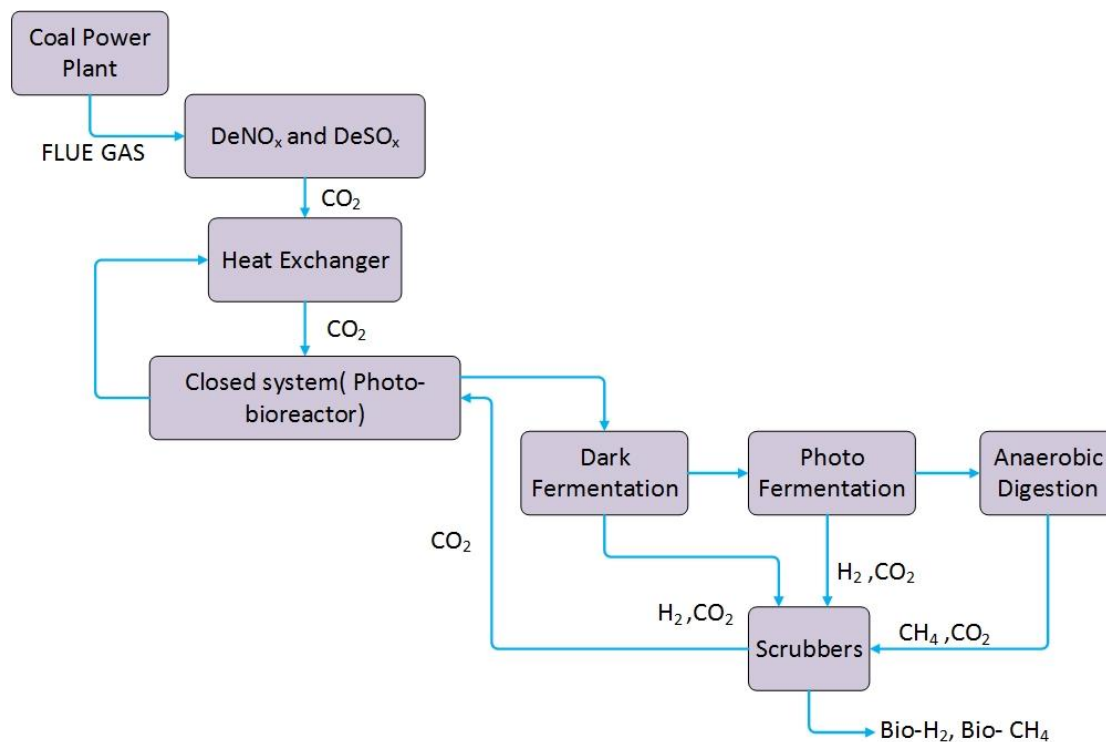


Figure 3. 2: Proposed system for production of gaseous fuel from micro-algal biomass produced using the flue gas of coal-fired power plants

3.2.2 Micro-algal biomass production from a 1GWe coal-fired power plant

3.2.2.1 CO₂ produced from a coal-fired power plant

The coal assessed is assumed to be bituminous with a 65% carbon content and an energy value of 24 GJ/t. Combustion of 2.82 Mt of coal per annum in a 1GWe coal power plant operating at an electrical efficiency of 35% and a capacity factor of 75% results in an emission of 6.77 Mt of CO₂ per annum (Box 3.1).

Box 3.1 CO₂ emissions from a 1GWe coal fired power plant

Assumptions

Bituminous coal at 65% carbon content and an energy value of 24 GJ/t combusted at an electrical efficiency of 35%; capacity factor of 75%

Combustion Equation

C + O ₂	to	CO ₂
12 tonne carbon	to	44 tonne CO ₂
1 tonne of carbon	to	3.7 tonne CO ₂
1 tonne of coal	to	2.4 tonne CO ₂ (coal 65% carbon)

Coal power plant

At an electrical efficiency of 35%, 1 tonne of coal can produce 2.33MW_eh.

430 kg coal equates to 1MW_eh and produces 1.03 t CO₂.

A 1GWe plant operating at 75% capacity consumes 2.82 Mt of coal per annum and produces 6.77 Mt of CO₂ per annum

3.2.2.2 Micro-algae produced from CO₂ emissions from a coal-fired power plant

Carbon constitutes 36-65% of dry algal biomass [6, 9, 22]. The ratio of (volatile solids) VS to total solids (TS) is 0.8. The analysis assumes that 1kg of CO₂ yields 0.5 kg of volatile solid algal biomass. A carbon capture efficiency of 80% and 50 % has been used for closed and open systems respectively. Thus, the closed system generates (0.5 kg VS * 80% capture of 6.77 MtCO₂/a) 2.69Mt VS while the open system generates (0.5 kg VS * 50% capture of 6.77 MtCO₂/a) 1.68 Mt VS (Table 2).

Photo-bioreactors have higher productivities than raceway ponds hence they occupy smaller areas making them more suitable for installation near large power plants. Photo-bioreactors can be flat plate or tubular [41, 42]. Micro-algal production and the land footprint of the different systems of cultivation have been calculated in Table 3.2. The areal productivities of the system are a function of the micro-algal species under cultivation, CO₂ fixation rate, method of mixing employed and mass transfer of CO₂. For example the tubular PBR has an areal productivity of 0.048 kg/m².d (or 0.48 t/ha.d). The area required for 2.69 Mt VS per

annum (or 7,370 t VS per day or 9,213 t TS per day) is 19,192 ha (Table 3.2). The open racing pond requires 52,303 ha. It is expected in the future that innovative reactor design with high biomass productivity can further reduce the land footprint.

Table 3. 2: Production per annum and foot print of micro-algal cultivation systems.

Method of cultivation	Micro-algae produced (Mt VS) ¹	Type of bioreactor	Areal productivity (kg/m ² . d) ¹	Area occupied (ha) ²	Reference
Closed system	2.69	Tubular	0.048	19,192	[43]
	2.69	Flat-Plate	0.027	34,094	[41, 42]
Open system	1.68	Raceway pond	0.011	52,303	[41]

1: Values from literature.

2: Calculated values

3.2.3 Energy return and carbon emissions allocation for the proposed system

Biological capture of CO₂ and the subsequent dark fermentation, photo-fermentation and anaerobic digestion of micro-algae species can yield an energy return of 35 % of the primary energy in coal for a closed system such as tubular photo-bioreactor (Box 3.2). This is sufficient fuel to fuel 600,000 cars per annum. For a tubular bioreactor this equates to 31 cars fuelled per hectare. This is a very high return especially considering that agricultural land is not required. For a closed system one tonne of coal can generate 8.4 GJ of renewable gas. One tonne of VS micro-algae can generate 8.8 GJ of renewable gas.

Box 3.2. Energy return from micro-algae using a three-stage sequential dark fermentation, photo-fermentation and anaerobic digestion of *Chlorella pyrenoidosa*

Gaseous fuel yields from CO₂ from coal combustion (using data from [12] as in Table 1)

1 tonne of coal can give 0.96 t VS of micro-algal ($2.4 \text{ tCO}_2 * 0.8 * 0.5 \text{ t VS/ t CO}_2$)

This corresponds to: 2 GJ H₂ gas ($198.3 \text{ m}^3 \text{ H}_2/\text{t VS} * 0.96 \text{ tVS} * 10.78 \text{ MJ/m}^3$)

& 6.4 GJ CH₄ gas ($186.2 \text{ m}^3 \text{ H}_2/\text{t VS} * 0.96 \text{ tVS} * 35.8 \text{ MJ/m}^3$)

Energy Return:

8.4 GJ renewable gas / 24 GJ = 35% energy return on the primary energy in the coal.

1 t coal produces 2.4 t CO₂, 0.96 tVS micro-algae (80% capture) & 8.4 GJ renewable gas.

1 t coal produces 2.4 t CO₂, 0.6 tVS micro-algae (50% capture) & 5.28 GJ renewable gas

Thus 1 t VS micro-algae produces 8.8 GJ of renewable gas

1 T CO₂ can produce 0.4 tVS micro-algae and 3.52 GJ (at 80% capture)

1 T CO₂ can produce 0.25 tVS micro-algae and 2.2 GJ (at 50% capture)

Cars powered:

A VW Passat consumes 4.4 kg/100 km or 6.6 m³ of bio-methane/100 km

If a car travels 16,700 km per annum it uses 39.5 GJ of CH₄

A 1GW_e plant consumes 2.82 Mt of coal per annum and can produce 23.7 PJ of gas.

600,000 cars powered by renewable gas

3.2.4 Energy consumption and carbon emissions of the proposed system

Thus, far the paper has considered gross energy return in the produced gaseous fuel generated through micro-algae cultivated in capturing CO₂ from coal fired power plant. This paper will not undertake a detailed analysis of all energy inputs or parasitic energy demand of the whole system as these are unknown for the three-stage sequential gaseous fuel production process as they are not yet commercialised. The paper provides a perspective on the relative differences in energy input in the micro-algal cultivation systems. Power consumption for pumping has been considered as the main energy input. This energy input if derived from a fossil fuel (such as coal) will reduce the net energy of the system and will create carbon emissions in capturing carbon.

3.3 Discussion of Results

The crux of the paper deals with the production of gaseous fuels from micro-algae that is grown using the carbon emissions from a coal fired power plant. However, it is observed from Table 3.3 that the energy output is higher for the closed systems than the open systems because pumping requirements are far higher in the closed systems especially in the tubular PBR. The ratio of the energy output to the energy input is 0.71 for the raceway pond; for each unit of energy put into the system only 0.71 units of energy leave the system. This drops to 0.05 for tubular photo PBR systems. At present level of technology, it may be said that the tubular PBR is not sustainable from an energy balance perspective. The energy input required to cultivate the micro-algae by carbon capture (GJ/tonne of CO₂) is significant, especially for the closed systems. Capture by flat plate and tubular PBR is 2.2 and 19 times more energy intensive respectively, when compared to chemical capture by Mono-ethanol amine (3.7GJ/tCO₂). Raceway ponds consume less energy than Mono-ethanol amine.

From a carbon perspective raceway ponds emit 31% of the carbon captured. This rises to 700% for the tubular PBR. Tubular PBRs because of their high pumping requirements are energy intensive, carbon intensive (if electricity from coal is used), as well as expensive. This negates the high biomass productivity per unit area of the tubular PBR system (Table 3.2).

Table 3. 3: Comparative analysis of micro-algal carbon capture and energy return using three different cultivation systems

Parameter	Raceway Pond	Flat Plate PBR	Tubular PBR
Energy output (GJ/tCO ₂) ¹	2.20	3.52	3.52
Volumetric productivity (kg/m ³ . d) ²	0.035	0.26	1.535
Power consumption (W/m ³) ²	4	50	2500
Energy input (GJ /t algal VS) ³	12.34	20.78	175.90
Energy input (GJ/tCO ₂) ⁴	3.09	8.31	70.36
Net energy ratio (NER); Ratio of energy output to energy input ⁵	0.71	0.42	0.05
Carbon emissions (t/tCO ₂ captured) ⁶	0.31	0.83	7.04

1: from Box 3.2

2: Values from literature [41, 43, 44].

3: Determined by dividing power consumption by (volumetric productivity* volatile solids to total solids ratio (0.8)). The calculation precedes as follows making allowance for the correct units (W/m³ / kg/m³. d = W/m³ * m³. d/kg = J.d/kg.s). For a raceway pond: (4 W/m³ * 24 h/d * 60m/h * 60 s/m) / (0.035 kg/m³. d) = 9,874,286 J/kg TS = 9.9 GJ/t TS = 12.34 GJ/tVS

4: Determined by multiplying values obtained in previous row (described in point 3) by conversion rate of CO₂ to micro-algal volatile solids (0.5), then by capture efficiency. For raceway ponds: 12.34 GJ/tVS * 50% conversion to microalgae VS = 6.17 GJ/ t CO₂ = 3.09 GJ/t CO₂ (50% capture efficiency)

5: NER: (Energy output (GJ/t CO₂)) / (Energy input (GJ/tCO₂)).

6: Determined by multiplying (Energy input (GJ/tCO₂)) by carbon emissions per tonne of coal (2.4t CO₂/t coal), divided by the primary energy content of coal (24 GJ/t)

The variation of micro-algal productivity has significant impact on NER (Figure 3.3) as well as carbon emissions of raceway pond (Figure 3.4). Passell et al. [45] suggested a low productivity of 0.003 kg/m². d (or 0.01 kg/m³. d) based on the measured productivity for year-around. This leads to a very low NER value of 0.20, which indicates energy input is much higher than energy output in micro-algal cultivation. The carbon emissions of 1.08 t/tCO₂ are also high, which indicate more carbon dioxide is produced during micro-algal cultivation process. While Delrue et al. recommended a relative high productivity of 0.03

kg/m².d (or 0.1 kg/m³.d), resulting in significantly increasing in the NER (2.04) and decreasing in the carbon emissions (0.11 t/tCO₂) [46]. A higher productivity of 0.128 kg/m³.d suggested by Stephenson et al. can further improve the NER to 2.61, and reduce the carbon emissions to 0.08 t/tCO₂ captured [23].

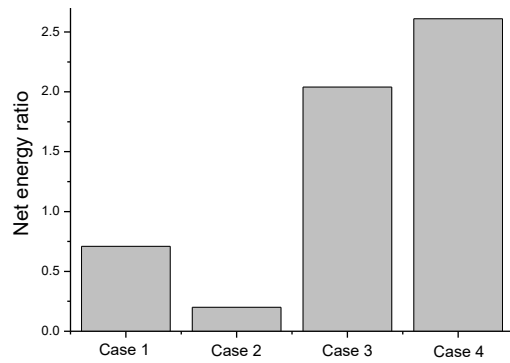


Figure 3. 3: Net energy ratios based on micro-algal productivities. Case 1: Jorquera et al. [41], Case 2: Passell et al. [45], Case 3: Delrue et al. [46] and Case 4: Stephenson et al. [23].

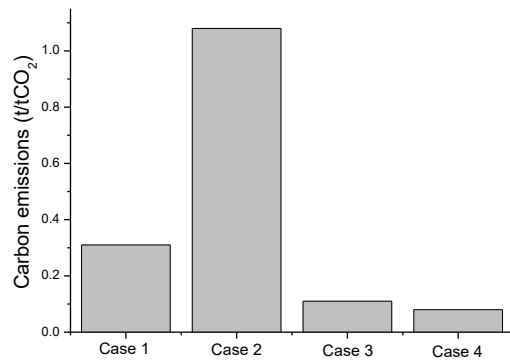


Figure 3. 4: Carbon emissions based on micro-algal productivities. Case 1: Jorquera et al. [41], Case 2: Passell et al. [45], Case 3: Delrue et al. [46] and Case 4: Stephenson et al. [23].

This analysis is meant as a perspective. It has examined the broader concept big picture. It has generated the potential energy returns in the form of gaseous fuel depending on the cultivation technology. Gaseous micro-algal fuels have benefits over biodiesel production as the considerable parasitic energy demand in drying the algae and extracting the lipids is not required. However, energy required to operate the three-step sequential process and energies in pre-treatment of the substrate have not been assessed. These steps will lower the energy return and increase the indirect carbon emissions of the system. A detailed life cycle analysis including energy and carbon analysis for each step of the proposed idea should include the following.

- Photo-bioreactors have high installation and operating costs;
- The carbon footprint associated with the construction and operation of the renewable gas production system;
- The electricity required to operate photo-bioreactors, particularly to bring about effective light utilization in the reactor for better micro-algal growth is significant, and should come from a carbon-free source;
- Although open systems are cheaper, they have high downstream processing costs and they suffer from contamination and require high water use.
- Analysis of the effects of diurnal variations and geographical conditions on algal growth need to be studied as this will result in a large variation in the yields reported

Micro-algal cultivation has the potential to combine with various combustion systems for CO₂ capture. The land footprint can be significantly reduced in a small combustion system (such as wood chip boiler). Alternatively, micro-algae can also be used in biogas upgrading by CO₂ removal to meet vehicle fuel standard (CO₂<3%) [47]. A recent study proposed an integrated system comprising biogas production and upgrading by micro-algae [48]. CO₂ in

produced biogas (ca. 40%) is efficiently removed by micro-algae via a carbonate/bicarbonate cycle. The accumulated micro-algal biomass can be used as co-substrates for further biogas production. This system has significant advantages in reducing parasitic energy demand in biogas upgrading, minimising CO₂ emission, and enhancing energy output of gaseous fuels [48].

3.4 Conclusions

The abundance of coal on the planet suggests that not combusting this coal is unlikely. Micro-algae may be used to capture the carbon and further be used to produce a gaseous biofuel with an energy content 35% of that of the coal. Cars may be powered at a rate of 31 per hectare. Of issue is the scale and the parasitic energy demand. A 1GWe coal fired power plant requires a micro-algal cultivation system occupying between 19,200 ha for a tubular PBR and 52,303 ha for a race pond system. The ratio of energy output to energy input is less than 1 for all the three cultivation systems assessed.

Despite this micro-algal carbon capture continues to gain attention. Increasing biomass productivity along with innovative photo-bioreactor design can lead to improvement in energy balance. Complete sequestration of CO₂ requires that the micro-algae are buried and not processed to produce combustible fuel.

At the current level of technology raceway ponds seem to be the only option for cheap and low carbon intensive micro-algal cultivation and carbon capture.

Acknowledgements

This research is funded by the Science Foundation Ireland (SFI) centre MaREI (12/RC/2302).

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4.Seaweed Biofuel derived from Integrated Multi-trophic Aquaculture

Seaweed Biofuel derived from Integrated Multi-Trophic Aquaculture

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Abstract

Aquaculture contributed 23.8 million tonnes of aquatic algae globally in 2012. Increasing consumption of seaweed (as food, for the production of hydro-colloids, and for production of third generation biofuels) will lead to an upward trend in its production and cultivation.

Aquaculture contributed 66.6 million tonnes of fish in 2012, 42 % of global production. Fish demand globally is rising to meet food and nutritional requirements; aquaculture for fish will grow. However, fish farms are marred by criticism of pollution caused by discharge of waste. Integrated multi-trophic aquaculture can reduce pollution through co-culture of several species such as seaweed and mussels that utilise waste disposed from fish farms for their growth and development.

A model is investigated which would provide 1.25% of energy in transport in the EU from seaweed. This would involve annual production of 168Mt of seaweed (more than present world harvest) integrated with 13 Mt of farmed salmon. The model proposes 2603 anaerobic digesters, each treating 64,500t/a of *S.latisma* in coastal digesters adjacent to natural gas infrastructure for downstream use in natural gas vehicles.

Keywords: seaweed; hydro-colloids; gaseous biofuel; integrated multi-trophic aquaculture; bio-hydrogen; bio-methane.

4.1 Introduction

4.1.1 The market for seaweed

Seaweed (macro-algae) is extensively used as a food in several countries including China, Japan and the Republic of Korea. In the last decade seaweeds, have been used to produce hydrocolloids in the food processing and cosmetics industry. Recent applications of seaweeds include in the field of bio-catalysis, bio-plastics, pharmacology and textiles [1]. The level of use of seaweed is excessive for natural stocks; hence close to 90 % of the seaweed used today comes from aquaculture [1]. There has been a significant increase in the production of farmed aquatic plants. The FAO reported a production of 15.8 million tonnes (wet weight) of aquatic plants in 2008 from aquaculture; 99.6% of this production is seaweed [2]. By 2013 the aquaculture harvest rose to 26.1 million tonnes of aquatic plants; again, the majority of which is seaweed [3]. This is a 65% increase in 5 years. In 2013, China was responsible for 13.5 million tonnes of this harvest [3]. The seaweed industry is valued at US\$ 5.5-6 billion annually [4]. Products for human consumption account for US\$ 5 billion of this [1, 5]. Hydrocolloids are substances, which form gel in water. In the food industry, there are used to bind food proteins in the dairy and meat industry. Seaweeds can be a vegetarian substitute for gelatine. The hydrocolloids industry produces alginates, agar and carrageenan from seaweed; this industry was worth US\$ 600 million in 2003 and increased to US\$ 1156 million in 2014 [1, 6]. This is an increase of 92.6% in 11 years.

4.1.2 The market for seafood

By 2050 our planet will be home to close to 9.6 billion people [7]. More food and nutrition will be required. Most importantly an adequate amount of protein will be necessary to prevent malnourishment. Meat protein is increasingly being used as a source of protein but it is unsustainable in the long run as the amount of CO₂ liberated per kg of edible meat is highest for cattle meat (30 kg CO₂/kg edible meat) and is the least for farmed fish (2.9 kg CO₂/kg edible meat) [8]. Globally around 158 Mt of food fish was produced in 2012; this

includes finfish, crustaceans, molluscs, amphibians, sea squirts and edible jellyfish. Aquaculture contributed 42 % to the total production of food fish in 2012; the remainder was supplied by capture production [3].

Protein from fish contributed 16.7% to the global animal protein intake in 2010, with 150 g of fish being sufficient to meet more than half of an adult's daily protein need [3, 9]. In 2012, 136.2 Mt of food fish was utilised for human consumption with an extra 21.7 Mt used for non-food uses, such as fish oil and fish feed used in aquaculture [3].

Global aquaculture (including food fish and aquatic plants) attained an industry value of US\$144.4 billion in 2012 and produced 66.6 million tonnes of farmed food fish, with farmed finfish accounting for two-thirds of the production [3, 10]. Salmon trade (both wild and cultivated) has increased considerably and contributes 14% to world fishery trade. Salmon and trout aquaculture is increasing in parts of North and South America (Canada, Chile) and Northern Europe; Norway leads the production of Atlantic Salmon [3, 8, 10, 11].

Europe is the largest market for fish and fishery imports with a value of US\$ 24.9 billion in 2012 (excluding intraregional imports). Europe is responsible for 23 % of the world imports of fish. Efforts are being made to make aquaculture more economically viable to reduce the burden of imports [3, 12].

4.1.3 Role of Integrated Multi-Trophic Aquaculture (IMTA)

IMTA is one of the most scientifically promoted methods of removing wastes from fish farms and has been used by Asian countries for centuries. It is now gaining importance as a method to reduce the ill-effects of fish farms (including inland and marine aquaculture) especially the discharge of inorganic nitrogen that is responsible for water eutrophication [13]. The basic concept of IMTA involves two levels: a Fed Trophic level (FTL) and an Extractive Trophic level (ETL). The FTL species may be Salmon or Trout (usually a carnivorous species). This species is the primary product being cultivated and is generally fed with fish processing

wastes or fish oil. The ETL species can be further divided as inorganic extractive species (such as seaweed) and organic extractive species (such as shellfish) [14-16]. The nutrient rich waste that is discharged by the fish farms is sequestered by these extractive species. The dissolved nutrients (containing nitrogenous compounds and phosphates) are absorbed by the inorganic extractive species (aquatic plants including seaweed). The floating and suspended particulate matter released is eaten by organic extractive species such as mussels, sea urchins and sea cucumbers [17-19].

4.1.4 Requirement for advanced biofuels such as sourced from seaweed

On the 24th February 2015, a press release from the Environment Committee of the European Parliament concluded that biofuels from seaweed or certain types of wastes should contribute at least 1.25 per cent of energy consumed in transport by the year 2020 [20]

Biofuels from seaweed is an emerging area of research for both liquid and gaseous biofuels [21]. It could not be said that there is any consensus on what the seaweed biofuel system would look like. What would be the species of seaweed? Would it be cast seaweed, or sub tidal seaweed? Would it be sourced from natural or cultivated stocks? Would the biofuel be liquid or gaseous? Whatever the system is, it is a massive task to generate 1.25% of energy from transport by 2020 from seaweed.

4.1.5 Objectives

This chapter presents a perspective on a seaweed biofuel system based on co-location of farmed fish and seaweed in an integrated multi-trophic aquaculture system. An objective is to suggest the resource of seaweed required to satisfy 1.25% of energy from transport by 2020 in the EU.

4.2 Fish farms, seaweed and gaseous biofuel production.

4.2.1 Salmon production and IMTA

Around 60 % of the global salmon production comes from salmon farms [8, 22]. Farmed Atlantic Salmon dominates the farmed salmon market with a share of more than 90 % and contributes more than 50% to the global salmon market [23]. The total supply of farmed Atlantic Salmon in 2013 was 1.84 million tonnes HOG (head-on-gutted) [8, 23]. Atlantic Salmon production is largely a function of seawater temperature and hence only selected coastal regions, where the water temperature is between 8 and 14 ° C is considered optimal for salmon growth and production. The main regions for production are around the coast of Norway, Scotland, Canada and Chile; in these areas certified licenses are required for farming as well as for catch production [8, 24]. A few studies have been carried out on bio-extraction by seaweed of carbon and nutrients excreted from fish farms [17]. Table 4.1 gives an overview of results obtained at field scale as well as laboratory studies to determine the nutrient sequestration capacity of certain seaweeds. Various factors such as water temperature, currents, light hours, seeding and stocking density of the seaweed affect the productivity of such a system [25].

Table 4. 1: Seaweed cultivation in Integrated Multi-Trophic Aquaculture

Fed Trophic level species	Seaweed cultivated	Reference
Sea bass (<i>Dicentrarchus labrax</i>), Turbot (<i>Scophthalmus rhombus</i>), Senegalese sole juveniles (<i>Solea senegalensis</i> Kaup)	A productivity of 23 g/m ² /day (dry weight) for <i>Gracilaria vermiculophylla</i> was achieved with a nitrogen removal capacity of 1.3 g/m ² /day.	[25]
Atlantic Salmon (<i>Salmo salar</i>)	Mean weight ratios of 6.7:1 and 12.9:1 for <i>Alaria esculenta</i> and <i>Saccharina latissima</i> were required to sequester nitrogen excreted per unit weight of salmon	[17]
Salmon farms located near Chile	A productivity of 53 g/m ² /day (fresh weight) for <i>Gracilaria chilensis</i> was achieved with a nitrogen removal capacity of 9.3 g/m for long line cultivation.	[26]
Atlantic Salmon	<i>Palmaria palmata</i> and <i>Saccharina latissima</i> were grown at a productivity of 180 t/ha/a and 220 t/ha/a and removed ca. 12 % and 5 % of nitrogen released by about 500 tonnes of fish over a period of 2 years.	[27]

4.2.2 Gaseous biofuels from seaweed

Gaseous fuels such as biomethane and biohydrogen can be produced from seaweed via thermochemical or biological processes. The ash content of seaweed is higher (ca. 15-30% dry matter basis) [28, 29] than terrestrial biomass (ca. 5-10 % dry matter basis) [30]. High ash content is a hindrance if used in thermal processes such as pyrolysis and gasification as ash causes fouling and slagging [31]. Hence seaweed may be more suited to anaerobic digestion.

Table 4.2 gives the biomethane yields for a selection of seaweeds. A study was also conducted on the methane potential of the sludge obtained post alginate extraction from seaweed yielding 100-150 L CH₄ /kg VS [32]. A recent study investigated the yield of hydrogen and methane from seaweed (*Laminaria japonica*) using a two-stage system (dark fermentation followed by anaerobic digestion). Yields of 71.4 ml H₂/g TS and methane yields of 309 ml CH₄ /g COD were obtained [33].

Table 4. 2: Biomethane potential of selected seaweeds

Type of seaweed	Methane yield L CH ₄ /kg Volatile solids (VS)	Reference
<i>Ulva lactuca</i>	271	[34]
<i>Laminaria digitata</i>	238	[35]
<i>Saccharina latissima</i>	256	[36]
	340	[37]
<i>Ascophyllum nodosum</i>	110	[38]
<i>Gracilaria vermiculophylla</i>	295	[39]

4.2.3 Potential resource of seaweed biofuel associated with a fish farm.

Figure 4.1 provides a concept of the proposed Fish to Fuel model. Depending on the composition (and hygiene) of the seaweed, it can be used for food and hydrocolloid production or biofuel. In some cases, where the cost of fish feed is expensive, operators of salmon farms may prefer to use the produced seaweed as fish feed.

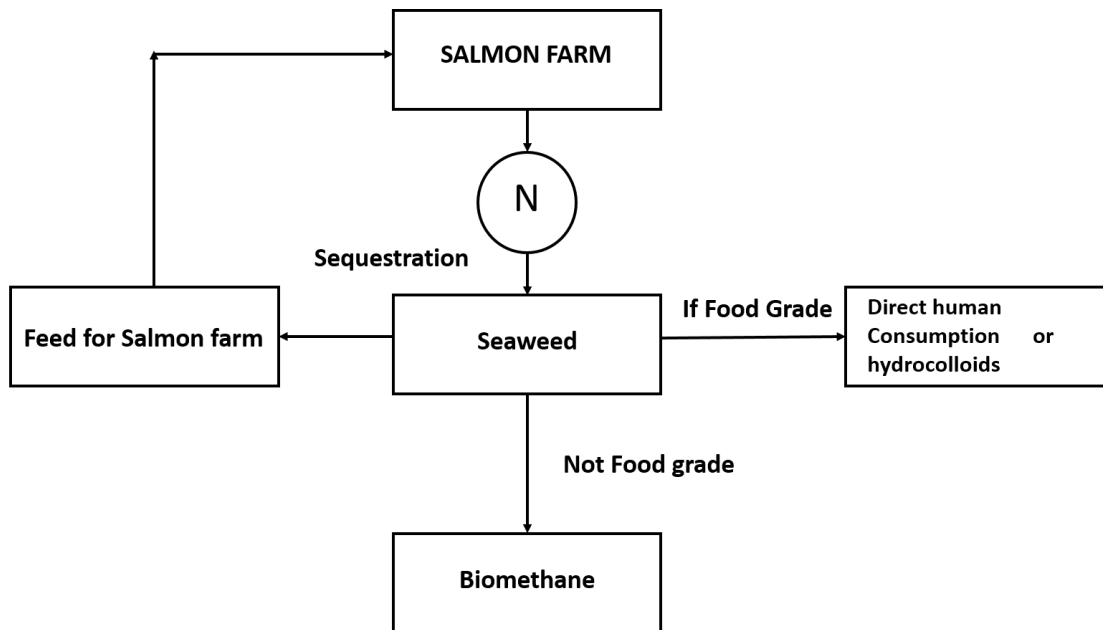


Figure 4. 1: Fish to Fuel Model

The average weight of an Atlantic Salmon after two years of growth at sea is in the range 3.6-5.4 kg [40]. The amount of nitrogen excreted per kilogram growth of Salmon is 29.49 g; this can be sequestered by 12.9 kg of *Saccharina latissima* (wet weight) [17]. Using a methane yield of 340 L/kg VS for *S. latissima* (Table 2) the resource of seaweed biomethane from a 5000-t salmon farm can be assessed as 79,216 GJ (Box 1).

In 2012, the total energy consumed in transport in the EU was of the order of 16.5EJ [41].

If advanced biofuels from seaweed are to satisfy 1.25% of this energy, then 206 PJ of transport biofuel is required per annum. In Box 4.1 it is shown that 5000 t of salmon can generate 64,500t of *S.latisma* or 79.2 TJ of biomethane. Based on this model 168 Mt of seaweed would need to be digested by 2020, in 2600 anaerobic digesters, each treating 64,500 t ww of laminaria per annum; at present the EU has approximately 9,000 digesters. The distribution system would be the existing natural gas grid. The vehicles would be natural gas vehicles (NGVs) of which there are over 16.7 M in operation in 2012 [<http://www.iangv.org/current-ngv-stats/>]

Thus, based on this model, the EU would need 13 million tonnes of salmon associated with the production of 168 Mt of seaweed. To put this in context the total supply of farmed Atlantic Salmon in 2013 was 1.84 million tonnes HOG (head-on-gutted). The world harvest of farmed fish was 66.6Mt in 2012. Aquaculture contributed 23.8 million tonnes of aquatic algae globally in 2012. A considerable ramping up of aquaculture is required for the EU to provide transport biofuel from seaweed.

Box 4.1: Seaweed biofuel system allowing production of 1.25% of energy in transport in the EU

Relationship between salmon and seaweed

A **5000 t salmon fish farm** produces **150t of nitrogen**
 [29.49g nitrogen excreted per kg of salmon]
 150 t of nitrogen allows production of **64,500 t (wet weight) of *S. latissima***
 [12.9 kg of *S.latissima* produced per unit weight of salmon]

Relationship between seaweed and biomethane

Biomethane production from 64,500 t (ww) of *S. latissima* = **2,212,737m³ CH₄**
 [64,500t (ww) *0.1009 (% VS)* 340 m³/t VS)]
 This scale is equivalent to a 1MW_e digester system (at 40% electrical efficiency).

Scale of industry required to satisfy 1.25% renewable energy in transport in the EU

Energy produced in seaweed biomethane from 5000 t of salmon = **79,216 GJ**
 [2,212,737 m³ * 35.8 MJ/m³]
 1.25% of energy in transport in the EU equates to 206 PJ
 2603 seaweed digesters each digesting 64,500 t ww of *S.latissima*, produce 16.5 EJ

Model of seaweed biofuel system

The model proposes that seaweed is harvested in late summer when the biomethane potential is highest. The seaweed is ensiled on shore adjacent to a coastal digester and to the natural gas grid. The biogas from the seaweed is upgraded to biomethane (methane composition of 97% plus) and injected to the natural gas grid. The Alternative Transport Fuel Directive stipulates that compressed natural gas service stations are situated no further than 150km distant in the EU by 2025 (<http://www.eubusiness.com/topics/transport/clean-fuels>)

4.2.4 Blue growth and blue carbon

The nutrient load of the 5000-t salmon farm is equivalent to the sewage released by a community of 37,500 people; 4 kg of nitrogen is excreted by an average human being per year [42]. Implementation of IMTA can have a three-fold benefit:

1. Excessive nutrient extraction/sequestration;
2. Co-production of diverse products whilst only feeding the main species (the lower trophic levels live off the waste from the fish farm);
3. Improved amenity of coastal habitat.

IMTA promotes high productivity of seaweed as there is a constant source of nutrients supplied. Similar to carbon credits, a nutrient credit system/trading is also implemented in countries (such as Sweden) for fish farms, thus increasing the total income generated by farmed fish aquaculture [25]. The release of wastewater can be taxed, such as employed in Denmark where charges of €4 per kg of N released, are in place [43]. If similar charges are imposed on salmon farms the use of IMTA can reduce the burden of such taxes. The nitrogenous wastes can be compared to valuable nutrients; nitrogen based fertilizers cost ca. €800/t [44]. Effective use of the coastal environment through IMTA concepts is classified as blue growth. Carbon sequestration in the marine environment is termed as blue carbon and is considered as an effective sink for carbon absorption [45].

4.3 Seaweed: Food versus Fuel debate

4.3.1 Use of seaweed for food

Irrespective of the nature and method of cultivation or harvest of seaweed, there could be a competition for the resource. Asian countries are the largest producers and largest consumers of seaweed. Unlike in the West, seaweed forms an important part of the cuisine in many Asian countries. In food circles *Laminaria* is known as kombu, *Undaria* is known as Wakame, *Porphyra* is known as Nori. Kombu, Wakame and Nori have sold at US\$ 2,800/dry tonne, US\$ 6,900/dry tonne and US\$ 16,800/dry tonne respectively [1]. Of the 220 species of

seaweed cultivated, *Laminaria*, *Undaria*, *Porphyra* contribute 74.8 % of total production [4]. There is an increasing market in Europe for seaweed as food. In Rogaland, Norway approximately 140 kg of *Ulva spp* is harvested by hand per annum; this is sold to restaurants at €50/kg ww [46].

4.3.2 Use of seaweed for industrial applications

Hydrocolloids from seaweed are a suitable alternative to synthetic gums, stabilisers, thickeners and gelling agents. Hydrocollids include for gelatin, xanthan, pectin, carboxy methyl cellulose, carrageenan, alginate, agar and guar; these are considered high value speciality chemicals. These are used in food products and pharmaceutical applications. Seaweeds have an asset value in industrial applications. *Laminaria hyperborea* can command a price of €23/tonne ww. *Ascophyllum nodosum* is sold at €50/tonne ww in Norway [46].

The world hydrocolloid market is expected to reach annual sales of US\$ 7911 million by 2019 [47]. The hydrocollid market is a competitor to seaweed biofuels.

4.3.3 Use of waste derived seaweed as fish feed

Seaweed that is grown using waste streams from integrated multi trophic aquaculture is a suitable feedstock for biofuel, as it does not directly compete with natural or farmed resources. Its primary function is to sequester nutrients from the waste secreted from fish farms and as such may not be seen as a high value food for human consumption commanding prices such as for Kombu (*laminaria*) of US\$ 2,800/dry tonne.

Fish feed and fish oil high in omega-3 fatty acids, are the preferred choice of feed for fish farms, especially for species such as salmon and trout. Fish feed has witnessed a considerable increase in price [3, 48, 49] and is responsible for 50-70 % of the production costs of fish farmers [50]. The prices of fishmeal and fish oil had increased to ca. US\$ 2000/tonne in 2013 and has remained around the same value in 2015 [3, 51]. Alternative sources of fish food

such as soymeal and corn meal have been used. Micro and macro-algae (seaweed) are also suggested to supplement the nutrient requirement of fish farms. Certain species of sea urchins, abalones and fish utilise seaweed as their source of food during the early stages of growth [52]. Hence the seaweed produced from fish farms may partly be used as feed for the organisms being cultivated.

4.3.4 Further Research

Much research is required on seaweed and biofuel production from seaweed. Technical and economic feasibility of offshore and onshore based IMTA systems is required. Offshore systems will require new infrastructure to be built (such as structural rigs); onshore systems require land. Detailed composition of the seaweed produced using IMTA is necessary. Life cycle analysis including for sustainability analysis for seaweed biofuel [52] is required to justify the benefits of this third-generation biofuel as compared to first (food crops) and second (lignocellulosic biomass) generation biofuel systems. Biorefinery systems, which include for biofuel production from the residues obtained after alginate and other high value products have been extracted, should be assessed [51].

Moreover, the sustainability of salmon farms may also be assessed. The production of farmed finfish is associated with many problems such as disease outbreak; that can also affect the wild species present in the natural water. Use of antibiotics, chemicals and steroids are damaging to the ecosystem, as are the high levels of nutrient discharge from the waste from fish farms [26, 53, 54]. Regulations will come into force, which will ultimately improve on the shortcomings of aquaculture [55-59] and may lead to IMTA and seaweed production becoming standard at fish farms.

4.4 Concluding remarks

Seaweed is a food and a versatile raw material. If advanced biofuels from seaweed are to satisfy 1.25% of energy in transport, the EU would need 13 million tonnes of salmon, generating 168 Mt of seaweed. The world harvest of farmed fish was 66.6Mt in 2012; aquaculture contributed ca. 23 million tonnes of seaweed in 2012. Natural stocks of seaweed cannot be involved in this increasing demand for seaweed. IMTA can improve the sustainability of fish farms, clean the waters of excess nutrients and supply seaweed as raw material for industry and as biofuel.

Acknowledgements

This research is funded by the Science Foundation Ireland (SFI) centre MaREI (12/RC/2302).

Glossary

Food Fish: Includes Finfish, Crustaceans, Molluscs, Amphibians, Sea Squirts, Edible Jellyfish.

Fish Feed: Food used for the growth of fish in farms (includes fish processing waste, soyfeed, seaweed)

Hydrocolloids: Colloidal particles that are hydrophilic polymers dispersed in water to form a colloidal solution.

Finfish, crustaceans, molluscs: Salmon, Shrimps, Mussels.

Extractive species: Species that derive nourishment from their environment, especially from the waste matter excreted by a higher trophic level in the surroundings.

Stabilisers: Additives added to food products to maintain their structure and to prevent emulsions from splitting into their individual components.

Feed conversion ratio: Measure of how effectively the feed is converted to animal body weight.

Protein retention: kg protein present in edible parts/kg protein in feed.

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5.Comparative study of single and two stage mono-fermentation of brown seaweed *Laminaria digitata*

Comparative study of single and two stage mono-fermentation of brown seaweed *Laminaria digitata*

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Abstract

This study contrasted single and two-stage mesophilic fermentation of *Laminaria digitata*. The two-stage system comprised a hydrolysis reactor (H₁) with a hydraulic retention time (HRT) of 4 days followed by two methanogenesis reactors M₁ and M₂ with HRT of 20 and 14 days respectively. The single stage reactor (M₃) had a HRT of 24 days. Specific methane yields of 176, 234 and 221 L/kg VS were obtained for M₁, M₂ and M₃. The methane concentration of the biogas was 22% higher for the two-stage system (58% to 61%) than the single-stage system (50%). Hydrolysis yielded a hydrogen yield of 26 L/kg VS. The two-stage system (H₁, M₂) provided an overall energy yield of 8.66 MJ/kg at a HRT of 18 days compared to 7.89 MJ/kg in the single-stage system with a HRT of 24 days. Thus two-stage system reduced HRT by 33% whilst improving the energy conversion by 9.8%.

Keywords: Seaweed; Methane; Biogas; Fermentation; Hydrogen; Hydrolysis

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5.1 Introduction

The sustainability of first generation biofuels derived from terrestrial crops (such as rapeseed biodiesel and maize ethanol) is in doubt and has raised ethical questions in the food fuel debate. Second generation biofuel feedstock tends to be highly ligno-cellulosic woody type crops; this increases the parasitic energy input for processing to suitable forms of biofuel (Allen et al., 2015). Marine biomass such as seaweed (also known as macro-algae) are considered third generation feedstocks for biofuels, as they do not compete with land based food systems, nor do they require any land for growth (in contrast with woody non-edible second generation substrates). Algae are a highly productive biomass and are easy to ferment as they contain no hard lingo-cellulosic material (Coelho et al., 2014).

However macro-algal based biofuel is at a very low technology level and there is a sparsity of literature on algal biogas. Feedstock procurement is an issue as excessive removal of natural seaweeds can cause environmental imbalance and habitat destruction. Cultivation is preferable but has some issues in the immaturity of the industry; sowing, harvesting, logistics of collection, storage, processing, and the high cost of all these steps can be challenging. Seaweed composition is also subject to seasonal variation in its composition (Tabassum et al., 2016c). Moreover there are certain kinds of seaweed that are more valuable and profitable for the production of value added chemicals (used in industrial gums, emulsifiers and hydrocolloids) than for biogas production (Hardouin et al., 2014; Fertah et al., 2014). Circular economy concepts may offer advantages to the seaweed biogas system. This includes integrated multi-trophic aquaculture, whereby cultivation of seaweed adjacent to fish farms can sequester nitrogenous compounds excreted by the fish increasing growth of the seaweed and reducing eutrophication of the marine environment (Abreu et al., 2011)

Seaweeds have no lignin hence are quite easily broken down by even mild processing temperatures. However, they contain very different polysaccharides such as alginate,

fucoidan, agar, mannose (van Hal et al., 2014). These seaweeds can contain cations such as calcium and magnesium that can reduce efficient degradation of the polysaccharides to their monomers. However, when anaerobic inoculum are acclimatised to seaweed feedstocks for a significant period of time, degradation of these complex carbohydrates is increased (Tabassum et al., 2016a). *Laminaria digitata*, which is a typical brown seaweed found in temperate oceanic waters, is comprised predominately of carbohydrates, with very low levels of proteins when compared to red or green algae (Hong et al., 2014). As a result, *L. digitata* is suitable for the production of biogas in the form of hydrogen and methane via fermentation.

Single stage anaerobic digestion of brown seaweed such as *Laminaria* spp. were conducted showing that particle size reduction yielded an increase in methane yield of upto 53% (Tedesco et al., 2014). Continuous prolonged periods of anaerobic digestion was also found to be conducive for *Laminaria hyperborea* (Hinks et al., 2013). Certain studies also reported low yields and unstable operation due to some inhibitory compounds such as polyphenolic pigments present in specific brown seaweeds. Certain seaweeds such as *Ascophyllum nodosum* are considered to be unfit for anaerobic digestion as their specific methane yields (SMY) are low, with levels of 47 L CH₄/kg VS recorded (Tabassum et al., 2016b). However, *A. nodosum* may be used as a source of pigments and alginic acid; it may also be used as a fertilizer. Since brown seaweeds tend to have good carbon to nitrogen (C:N) ratios in advance of 20:1, they can also be co-fermented with protein/nitrogen rich substrates like microalgae and dairy slurry to improve their methane yield (Herrmann et al., 2016; Tabassum et al., 2016a).

Two stage fermentation involves hydrolysis of an initial substrate in a separate reactor to volatile fatty acids (VFAs) at an optimum pH in the range 5 to 5.5 (Jung et al., 2011b; Kim and Kim, 2011). The VFAs are then fed to a second methanogenic reactor that produces biomethane with the aid of the methane archaea at a pH in the range of 7 to 7.5. The two-

stage process is seen as theoretically improving both the hydrolysis and methanogenesis stages as pH is optimised for these in different reactors. This is not the case for a single stage where the pH is in the range of 7 to 7.5 in a single reactor and as such hydrolysis is not optimised. The hydrolytic stage also results in the production of biogas free from methane and including hydrogen and carbon dioxide. This process is often referred to as dark fermentation (Krupp and Widmann, 2009; Levin and Chahine, 2010). Several types of feedstocks have been assessed for two stage digestion in the literature including: straw, grass silage and food waste; these substrates have been converted to biogas in laboratory two-stage processes with reasonably high yields (Browne and Murphy, 2014; Massanet-Nicolau et al., 2015).

Fermentative hydrogen production and two-stage fermentation of *Laminaria japonica* has been studied by Jung and co-workers (2011a; 2011b; 2011c; 2012). Optimised pre-treatments including for acid pre-treatment and high temperature treatments facilitated hydrogen yields of 159.6 L H₂/kg dry cell weight (Jung et al., 2011a). Liu and Wang (2014) investigated the effect of pH and mixed anaerobic bacteria on fermentative hydrogen production from *L.japonica*. Jung et al (2012) focused on continuous two stage digestion of *L. japonica*; in this study the effluent from the methanogenic stage was used as an alkali to maintain the pH of the dark fermentation reactor (Jung et al., 2012).

L. digitata is one of the dominant seaweeds in the north west Atlantic and is known for its high growth rate. The scientific literature records the effect of seasonal variation on its methane yield; August is the most suitable season for harvest in terms of biomethane potential (Adams et al., 2011; Tabassum et al., 2016c). *L. digitata* and micro-algae (*Chlorella pyrenoidosa*) were converted to biogas in a batch two-stage fermentation improving hydrogen and methane yields as compared to mono-fermentation of micro-algae (Ding et al., 2016). This chapter deals with the comparative study of a two-stage process and a single

stage system both digesting *L. digitata*. A scientific literature review indicates only one study on long term continuous digestion of *L. digitata* (Tabassum et al., 2016) and no study on two-stage continuous mono-fermentation of *L. digitata*. The innovation in this chapter is to fill the gap in the literature by assessing the following objectives:

- Compare the performance of a two-stage and single-stage anaerobic digestion of *L. digitata* based on process parameters including hydraulic retention time and organic loading rate.
- Assess the specific methane yields and biogas composition for the two systems.
- Assess the specific hydrogen yield and volatile fatty acid production of the hydrolysis reactor.

5.2 Material and Methods

5.2.1 Materials

The seaweed *L. digitata* was collected from the beaches of Cork in Ireland during the months of September and October (Herrmann et al., 2015). The biomass thus obtained was thoroughly washed under tap water to ensure the removal of sand and other forms of impurities in the surface, and then was processed to obtain a particle size of 4-5 mm using a Buffalo Heavy Duty Mincer CD400. The processed samples were subjected to proximate analysis. The seaweed was shown to have 18.44 wwt % (total solids or TS), 14.05 wwt % (volatile solids or VS), 81.56 wwt % (moisture) and an ash content of 22.84% (of Total Solids). The ultimate analysis gave the chemical composition as follows: C (36.01 %TS), H (4.59 %TS), N (1.32 %TS), O (35.24 %TS) and C: N ratio (27.3). A biomethane potential (BMP) assay generated a value of 305.2 ± 9.1 L/kg VS. The BMP of an internal standard such as cellulose (350.2 ± 12.7 L/kg VS) was also done to check the validity of the results as well as the proper functioning of the equipment.

Inoculum for the methanogenic reactors were passed through a 2-mm sieve and incubated for at least one week at 37°C under anaerobic conditions to reduce any residual gas production. The hydrolysis reactor was seeded with inoculum that was heat treated at 100°C to eliminate methanogenic archaea thus facilitating the dominance of hydrogen/hydrolysing bacteria.

5.2.2 Chemical and Biological Analyses

Gravimetric measurements such as TS and VS were obtained by weighing the sample residues that were dried for 24 hours at 105 °C and later burning the dried residue at 550 °C for 4 hours. Volatile Fatty Acids (VFA) were determined using a gas chromatograph (Agilent HP 6890 Series, Agilent Technologies, Santa Clara, CA, USA) equipped with a Nukol™ fused silica capillary column (Supelco, Bellefonte, PA, USA), argon as a carrier gas and flame ionisation detector. Gas samples were measured using a gas chromatograph (Agilent HP 6890 Series, Agilent Technologies, Santa Clara, CA, USA) equipped with a Hayesep R packed column and a thermal conductivity detector. The samples were premethylated as per ISO standards. The pH was measured using a Jenway 3510 pH meter.

Chemical composition such as elemental carbon (C), nitrogen (N) and hydrogen (H) were measured using an elemental analyser with thermal conductivity detector (CE 440, Exeter Analytical, Coventry, UK). The ratio of VFA to bicarbonate alkalinity, known as the (FOS/TAC), was determined by a two-point titration method using 0.1 N sulphuric acid titrated against the samples to endpoints of pH 5.0 and pH 4.4 applying a TITRONIC® Universal Automatic Titrator (SI Analytics GmbH, Mainz, Germany). Total ammonical nitrogen (TAN) was obtained using Hach Lange cuvettes (LCK 303 and LCK 311) and a spectrophotometer DR 3900 (Hach Lange GmbH, Dusseldorf, Germany). A biomethane potential (BMP) assay was evaluated on the substrate *L. digitata* using the automatic methane potential test system (AMPTS II, Bioprocess Control, Lund, Sweden). In this batch test 500 mL glass bottles were filled with inoculum and seaweed at an inoculum-to-substrate ratio of

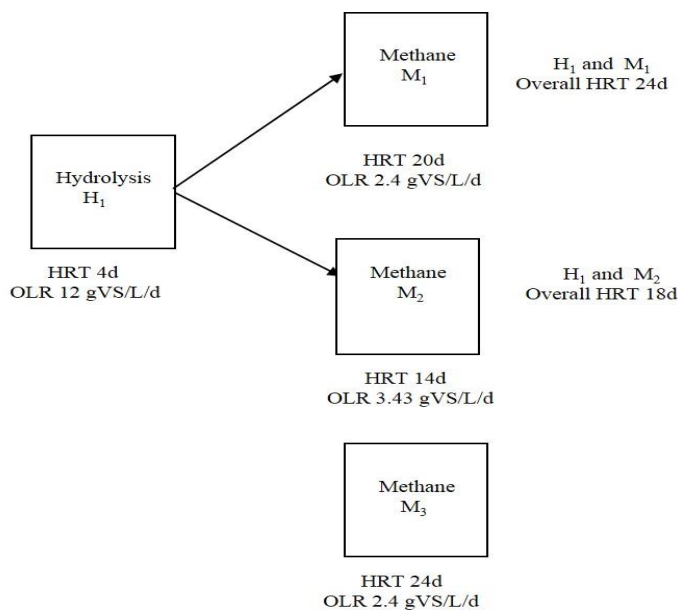
2:1 (VS basis) to occupy a final volume of 400 mL. The bottles were sealed and the headspace was flushed with nitrogen to maintain anaerobic conditions. These bottles were then incubated at 37°C for a period of 30 days in a water bath. The reactor contents were stirred semi-continuously at 30 rpm at intervals of 60 s. The same test was also conducted for the inoculum that acted as the blank sample. Biogas thus produced was scrubbed through a 3 M NaOH solution for the removal of CO₂ and other trace gases. The total volume of the scrubbed gas containing only methane was determined by an in-built wet gas flow measurement device. The volume of methane was normalised to standard temperature and pressure (standard atmospheric pressure, 0°C, dry gas). The final BMP of the seaweed was corrected for the methane produced by the inoculum (Allen et al., 2015).

5.2.3 Set-up and operation of the continuous reactors

Four PVC cylindrical reactors of total volume of 5 L with working volume of 4 L each were used. The reactors were maintained at a temperature of 37± 1°C by using a thermal water bath that circulated hot water through the heating coils that were mounted around the reactors. To maintain homogeneity of the reactors contents, vertical stirrers with upper and lower paddles were used. The reactors were fitted with appropriate tubings through which the produced biogas was measured using a tipping device that was connected to a computer (digital data logger, LabJack Lakewood, CO, USA), giving the actual volumes of gas produced. The measured gas was then collected in gasbags to determine its composition using the GC.

The working volume of the hydrolysis reactor was 2.5 L. The hydrolysis reactor uses considerably larger amounts of raw material as this reactor has far higher loading rates when compared to the methane reactors (Figure 5.1). Hydrolysis reactor (H₁) was fed with *L. digitata* initially at an organic loading rate (OLR) of 7 g VS/L/D with a hydraulic retention time (HRT) of 4 days. The HRT was maintained throughout by varying the dilution rate with

water when the OLR varied. Water was added to keep the volume of the substrate entering the reactor a constant that helped keep the HRT at 4 days. Because of this dilution salinity affects were not considered. This hydrolysis reactor operated for a month at this initial OLR to ensure sufficient digestate in the form of VFAs were produced. The digestate effluent rich in VFAs was divided between methane reactor (M_1) with a HRT of 20 days and an OLR of 1.4 g VS/L/d and methane reactor (M_2) with a HRT of 14 days and an OLR of 2 g VS/L/d. Together H_1 and M_1 formed a system with a combined HRT of 24 days and H_1 and M_2 a system with a combined HRT of 18 days. To assess the performance of these two-stage fermentation processes a single stage methane reactor (M_3) was initiated at an OLR of 1.4 g VS/L/d at an HRT of 24 days. The TS content of the reactors were kept under 8% to minimise any stirring issues as the OLR increases



Notes: H_1 and M_1 , H_1 and M_2 are two stage systems; M_3 is a single stage system. Loading rates and retention times are at the end of the experiment period

Figure 5. 1: Experimental design of the two-stage and single-stage fermentation systems.

The methane reactors were run for a length of time equivalent to a HRT and then the OLR of the H_1 reactor was increased from 7 to 9 g VS/L/d while the OLR of M_1 and M_2 were increased from 1.4 to 1.8 and 2 to 2.57 g VS/L/d respectively. The OLR of the single stage

methane M₃ system was also increased from 1.4 to 1.8 g VS/L/d to track and match the two-stage reactor system of H₁ and M₁, which has the same combined HRT of 24 days as that of M₃. The reactors were run for acclimatisation and process stability till the hydrogen composition in the hydrolysis reactor was at a constant value of approximately 20% and the pH range was between 5-5.5; the methanogenic reactors were acclimatised till a methane composition of approximately 48-50 % was achieved. The three methane reactors and the hydrolysis reactor were run for a commissioning period of 96 days to obtain the desired values of pH, VFA, methane yield and its composition as given in Table 5.1. The data in table 5.1 are average values noted during the commissioning period of 96 days. After commissioning the process was assessed for 50 days at final operating parameters of: 12 g VS/L/d for H₁; 2.4 g VS/L/d for M₁; 3.43 g VS/L/d for M₂; and 2.4 g VS/L/d for M₃

Table 5. 1: Average values and range of operating parameters during the commissioning period

Hydrolysis Reactor	OLR (g VS/L/d)	pH	VFA (mg/L)
H ₁	7	7-6.2	800-1500
	9	6-5.5	2600-5700
Methane Reactor	OLR (g VS/L/d)	% CH₄	CH₄ yield (L/kg VS)
M ₁	1.4	37	94
	1.8	49	150
M ₂	2	39	147
	2.57	56	221
M ₃	1.4	38	131
	1.8	51	211

5.3 Results and Discussion

5.3.1 Hydrolysis reactor

Seaweed is a complex substrate as compared to simple sugars that readily degrade in a hydrolysis reactor, leading to higher multiplication rates of hydrolysing bacteria. In such

cases pH, can drop quickly to the desired hydrolysis range of 5 to 5.5. However, since the seaweed *L. digitata* is a novel marine substrate; microbial fauna take a longer time to acclimatise and produce enzymes capable of breaking down the cell wall consisting of hetero polysaccharides made up of cellulose and alginic acid (Kerner et al., 1991). *L. digitata* is a brown seaweed that contains xanthophyll pigment fucoxanthin giving it its characteristic brown colour. Laminarin (made up of glucose), fucoidan (made up of fucose, xylose, mannose and galactose), alginate and mannitol are the unconventional carbohydrate molecules found in brown seaweed; these can be difficult to completely breakdown through microbial action of digestates associated with digestion of terrestrial agricultural feedstocks (Bidwell and McLachlan, 1985; Seyfried, 1996). However, as the OLR in the hydrolysis reactor was increased from 7 to 9 g VS/L/d a gradual pH reduction was observed to a range of 5.2 to 5.6. This is close to the ideal pH operating condition, which maximises VFA production. Along with the pH the biogas produced from the hydrolysis reactor was monitored. Initially the amount of hydrogen in the biogas was around 30%, which later reduced to ca. 20% with the increase in OLR. This whole period was considered as the start-up period of the hydrolysis reactor. When efficient VFA production and optimum pH levels were achieved then the OLR was increased to 12 g VS/L/d. At this loading hydrogen yields of 26 L/kg VS were achieved. This value is on par or even slightly higher than values obtained from terrestrial feedstock such as pre-treated sewage sludge (18.4 L H₂/kg dry solids) and wheat co-product (7.0 L H₂ /kg VS) (Massanet-Nicolau et al., 2015). However this value is less than values of hydrogen obtained from batch fermentation of steam pre-treated *L. digitata* of 83 L/kg VS (Xia et al., 2016).

5.3.2 Methane reactors and energy yields.

The methane reactors M₁ (HRT 20 d) and M₂ (HRT 14 d) were started at an OLR of 1.4 and 2 g VS/L/d. Since the OLR was higher in M₂ the amount of VFAs that it received from the

hydrolysis reactor was significantly higher than M_1 ; as a result, after the initial period of start-up and acclimatisation, the amount of methane produced in M_2 was higher than M_1 . At an OLR of 2.4 g VS/L/d (M_1) and 3.43 g VS/L/d (M_2), the methane yields for M_1 and M_2 were 176 L/kg VS and 234 L/kg VS respectively. With a short HRT of 14 d, M_2 performed better than M_1 as it can be observed that once enough VFAs are produced, a high OLR can be employed in the methane reactors to obtain better yields with short HRTs. This aspect is of great interest to industries as heating costs and operating costs can be minimised by short HRTs (Schievano et al., 2014; Ljunggren and Zacchi, 2010). However, reducing the HRT may lead to microbial washout and acidic pH if reactors are run for a very long time. Accumulated VFAs at higher OLRs can bring about microbial stress as maintaining the pH in the range of 7-7.5 can get increasingly difficult. Sufficient alkali addition (3M NaOH) was added to prevent the pH from going below 7.

Dried brown seaweed of the variety *L. japonica* was converted to methane through a two-stage process using the effluent from the hydrolysis reactor as influent to an anaerobic sequential batch reactor (ASBR) and an up-flow anaerobic sludge blanket reactor (UASBR); values of 200-300 L CH_4 /kg COD were obtained (Jung et al., 2012). The use of dried seaweed is not feasible at commercial scale as the energy demand associated with the drying process is very high.

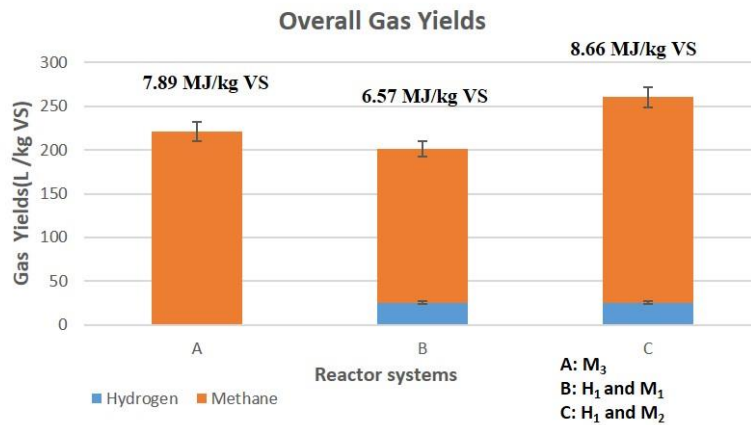
The single stage reactor M_3 (HRT 24 d) digesting *L. digitata* and not VFAs formed by the hydrolysis of *L. digitata* in H_1 performed on par with the two stage systems, with a methane yield of 221 L/kg VS at an OLR of 2.4 g VS/L/d. Indeed, M_3 performed better than the two stage-system of $H_1:M_1$ (with an effective HRT of 24 days (4d+20d)) as M_3 had a more stable pH (ca. 7.6) and had a slightly longer retention time. The performance of M_3 was almost on par with that of $H_1:M_2$ (with an effective HRT of 18 days (4d+14d)). However, M_3 operated at a lower OLR than M_2 . Seaweed has no lignin hence it does not require longer retention

times as opposed to grass and other lignocellulosic feedstocks that need anywhere between 25 to 30 days of HRT; two stage digestion reduces the HRT to less than 20 days as reported by (Massanet-Nicolau et al., 2015). Hence the retention times used in this study are based on literature and the chemical characteristics of the feedstock.

The methane composition of the biogas from the two stage systems were 58% and 61% for M_1 and M_2 respectively. These values are significantly higher when compared to the methane composition of 50% obtained from the single stage reactor. This may be cost effective in upgrading biogas to biomethane in that some of the work is already done through separation of some of the CO_2 production in the hydrolysis reactor. Thus, employing a two-stage system with the appropriate operating conditions and process stability can have the advantages of lower HRT (and associated cheaper and smaller reactors) and higher methane composition in the biogas (with cheaper upgrading systems) when compared to single stage systems.

Figure 5.2 gives the methane and overall energy yields of the reactor systems. Lower heating values of methane (35.8MJ/kg) and hydrogen(10.98 MJ/kg) were used to calculate the energy yields. An energy yield of 7.89 MJ/kg VS was obtained from the single stage system consisting of only methane production. However, energy yields for the two-stage systems also accounted for the hydrogen produced (although in very small quantities). The overall energy yield of the two-stage 18 d retention time ($H_1:M_2$) system was 8.66 MJ/kg VS, which was 9.8 % higher than the single-stage system. The overall energy yield of the 24-d retention time two-stage ($H_1:M_1$) system was comparatively lower (6.57 MJ/kg VS). Specific gas yields can be based on volatile solids as well as chemical oxygen demand. However, these two give slightly different values as volatile solids includes even refractory organics that cannot be degraded by microbes. The organic matter present is digested to produce methane, carbon dioxide and is also used to produce microbial cells and for their maintenance. Chemical oxygen demand is the amount of oxygen used to oxidize soluble and particulate

organic matter. Another way of expressing specific gas yield would be to use BOD, which is biological/biochemical oxygen demand. This test takes 5 days hence it is not used to calculate biogas yields.



Notes: System A: M₃ (mono-fermentation); system B: H₁+M₁ (two stage fermentation); system C: H₁+M₂ (two stage fermentation).

Figure 5. 2: Overall gas yields and energy yields of the different reactor systems.

5.3.3 Process parameters determining process stability.

The weekly average values of the main process parameters that indicate the stability of the reactors were recorded as given in Tables 5.2-5.5; these values are the weekly data for all the reactors in the final assessing period (recorded for 6 weeks). The loading conditions were as per figure 5.1. The hydrolysis reactor was at an OLR of 12 gVS/L/d and the pH had started to drop towards 5. However sufficient quantities of alkali (3M NaOH) were added daily to prevent the pH from going below 5. A pH value of less than 5 can lead to poor hydrolysis, and ethanol formation rather than VFA production (Fasahati and Liu, 2015). Table 5.6 gives the synthesis of the final values averaged over the final 6 weeks of operation with design conditions as per figure 5.1.

Methanogenic reactors are stable when there is a balance between VFA and the alkalinity present in the reactor. This balance prevents the pH from going below 7 and prevents failure of the methanogenic reactors. A FOS/TAC value between 0.2 and 0.4 is ideal. Reactor M₁ and M₃ operated at a lower OLR than M₂ and had a FOS/TAC value closer to 0.2. Whereas the M₂ reactor operated at a higher OLR and the FOS/TAC value was in the middle of the stable range. As a result, the pH values of the reactor M₂ was reduced as compared to reactors M₁ and M₃. NaOH was added on alternate days to prevent the pH from reducing any further.

TAN is also a key parameter in the normal functioning of methane archaea. As the TAN increases microbes cease to perform efficiently as high TAN levels cause a pH imbalance in the cellular structure and can easily penetrate the cell membrane causing dysfunction in the cellular transport of nutrients within the cell. However, alkalinity is the result of protein degradation as amino groups and ammonia are released, this balances low pH environment if excess VFA accumulation takes place in the reactor. *L. digitata* has a very low nitrogen content (1.32%). This is true for most types of brown seaweed such as *L. digitata*, *L. japonica* and *E. bicyclis*, which have low protein content (less than 12%) as compared to red (ca. 23%) and green (ca. 20%) seaweeds such as *Ulva lactuca* and *Gelidium amansii* (Hong et al., 2014; Shi et al., 2011) which have higher levels of nitrogenous compounds. Hence fermentation of brown seaweed would result in low levels of TAN in the reactor. In addition to this the reactor contents were very dilute as water was added to fix the retention time. As a result, the values are extremely low and didn't pose any process instability issues.

Table 5. 2: Weekly data for Hydrolysis reactor H₁ at OLR 12 gVS/L/d

PARAMETERS	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
ACETIC (mg/L)	2388.51±0.21	2266.63±.45	2139.67±.5	2378.51±.4	2588.43±.56	1888.67±.65
PROPIONIC (mg/L)	53.92±.013	51.46±.32	34.64±.28	43.92±.3	13.4±.32	93±.47
ISO-BUTYRIC (mg/L)	117.33±.01	114.77±.02	104.89±.03	111.33±.034	132.32±.035	167.7±.03
BUTYRIC (mg/L)	4225.24±.16	4103.69±.2	4123.90±.2	3225.24±.19	4285±.18	3125.78±.12
ISO-VALERIC (mg/L)	20.35±.01	21.65±.014	13.41±.015	22.35±.018	20.35±.013	25.78±.014
VALERIC (mg/L)	30.32±.02	31.82±.02	32.89±.03	30.32±.035	29.31±.04	30.32±.05
ISO-CAPROIC (mg/L)	0	24.45±.04	0	0	0	0
CAPROIC (mg/L)	126.20±.26	125.47±.3	122.78±.4	136.2±.32	176.34±.38	115.67±.33
ENANTHIC (mg/L)	26.53±.01	41.10±.02	24.89±.03	22.53±.04	16.34±.05	24.89±.01
TAN (mg/L)	10.69±.01	10.8±.03	9.89±.02	11.2±.03	12.4±.021	12.2±.022
pH	5.5±0.2	5.21±0.1	5.19±0.3	5.35±0.1	5.23±0.2	5.13±0.1
SHY (L H ₂ /kg VS)	32.67±2.1	28.6±3.4	22.4±1.3	24.48±1.4	23.54±2.6	22.12±2.1

Table 5. 3: Weekly data for Methane reactor M₁ at OLR 2.4 gVS/L/d

PARAMETERS	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
ACETIC (mg/L)	590.48±.65	588.01±.4	548.98±.35	589.67±.58	467.89±.3	578.54±.5
PROPIONIC (mg/L)	143.83±.01	143.13±.03	145.90±.034	132.78±.02	105.8±.03	134.78±.05
ISO-BUTYRIC (mg/L)	21.61±.23	21.59±.2	22.50±.32	18.65±.33	15.78±.4	28.9±.3
BUTYRIC (mg/L)	21.47±.12	0	23.82±.14	22.56±.12	0	45.8±.2
ISO-VALERIC (mg/L)	0	0	21.50±	19.78±	0	0
VALERIC (mg/L)	0	21.49±.32	0	0	17.89±.33	0
ISO-CAPROIC (mg/L)	0	0	0	0	0	0
CAPROIC (mg/L)	0	0	21.47±.3	16.89±.2	0	48.97±.35
ENANTHIC (mg/L)	26.12±.01	0	0	0	0±	0
TAN (mg/L)	248±.3	235±.32	245±.43	250±.2	256±.2	238.5±.25
pH	7.6±.5	7.4±.2	7.55±.5	7.33±.10	7.65±.4	7.53±.2
FOSTAC	0.34±.23	0.47±.4	0.36±.1	0.14±.1	0.13±.32	0.326±.2
% CH ₄	58.43±1.5	59.2±2.3	57.34±1.6	58.4±2.56	59.3±1.7	56.42±1.2
SMY (L CH ₄ /kg VS)	196.5±12.5	188.4±16.8	160.56±20.6	166.7±15.78	174.2±22	169.54±25

Table 5. 4: Weekly data for Methane reactor M₂ at OLR 3.43 gVS/L/d

PARAMETERS	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
ACETIC (mg/L)	118.61±0.43	122.55±.32	117.16±.35	104.3±.5	145.89±.44	102.3±.3
PROPIONIC (mg/L)	171.17±.02	174.12±.03	0	0	156.89±.02	112.67±.02
ISO-BUTYRIC (mg/L)	43.62±.2	36.54±.3	168.29±.36	123.87±.4	21.78±.3	12.78±.4
BUTYRIC (mg/L)	0	19.38±.12	33.54±.18	35.7±.2	18.97±.28	18.56±.2
ISO-VALERIC (mg/L)	19.38±.02	0	0	0	0	0
VALERIC (mg/L)	0	0	19.29±.15	15.89±.18	0	0
ISO-CAPROIC (mg/L)	0	0	0	0	0	0
CAPROIC (mg/L)	0	0	0	0	0	0
ENANTHIC (mg/L)	0	0	0	0	0	0
TAN (mg/L)	370±.25	365±.3	366±.41	368±.23	372±.25	369.6±.2
pH	7.4±.2	7.22±.3	7.38±.2	7.5±.3	7.25±.3	7.48±.1
FOSTAC	0.35±.1	0.22±.14	0.46±.2	0.37±.26	0.13±.2	0.361±.3
% CH ₄	59.85±2.3	57.65±1.4	64.77±1.43	60.4±3.1	63.1±1.56	62.4±1.4
SMY (L CH ₄ /kg VS)	242.67±20.2	238.6±18.3	235.78±13.4	230.8±10.5	236.1±12.3	222.79±20.3

Table 5. 5: Weekly data for Methane reactor M₃ at OLR 2.4 gVS/L/d

PARAMETERS	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
ACETIC (mg/L)	171.08±0.33	162.32±.42	157.46±.15	187.56±.56	123.89±.24	167.5±.36
PROPIONIC (mg/L)	48.49±.01	49.37±.03	48.47±0.02	0	37.56±.02	24.67±.01
ISO-BUTYRIC (mg/L)	33.81±.21	26.58±.43	28.32±.38	56.75±.3	23.67±.25	28.76±.4
BUTYRIC (mg/L)	0	19.04±.11	0	21.675±.17	0	
ISO-VALERIC (mg/L)	18.85±.01	0	19.08±0.03	0	14.56±0.02	23.6±0.03
VALERIC (mg/L)	0	0	19.29±.18	16.56±.2	0	0
ISO-CAPROIC (mg/L)	0	0	0	0	0	0
CAPROIC (mg/L)	0	0	0	0	0	0
ENANTHIC (mg/L)	0	0	0	0	0	0
TAN (mg/L)	265±.3	270±.35	280±.48	278±.21	276±.24	287.5±.2
pH	7.54±.3	7.68±.2	7.33±.1	7.55±.2	7.46±.2	7.68±.3
FOSTAC	0.2±.14	0.13±.2	0.44±.28	0.21±.1	0.32±.3	0.126±.17
% CH ₄	48.65±3.4	48.6±2	52.78±2.5	46.6±5.1	51.6±1.3	52.6±2.3
SMY (L CH ₄ /kg VS)	200±18.4	220±13.5	213.6±20.5	225.8±12.4	230.5±20.5	232.6±11.2

Table 5. 6: Final average values taken for all weeks

Reactor	pH	S.D.	FOS / TAC	S.D .	TAN (mg/L)	S.D .	VFA (mg/L)	S.D.	%CH ₄	S.D	SHY or SMY	S.D
H ₁	5.26	0.12	-		11.19	0.87	6511.71	611.50	-		26	3.8
M ₁	7.51	0.11	0.29	0.12	245.41	7.02	767.77	74.35	58	1.01	176	12.54
M ₂	7.37	0.10	0.31	0.10	368.43	2.39	318.887	40.96	61	2.33	234	6.30
M ₃	7.54	0.12	0.23	0.11	276.08	7.17	251.61	26.34	50	2.31	221	11

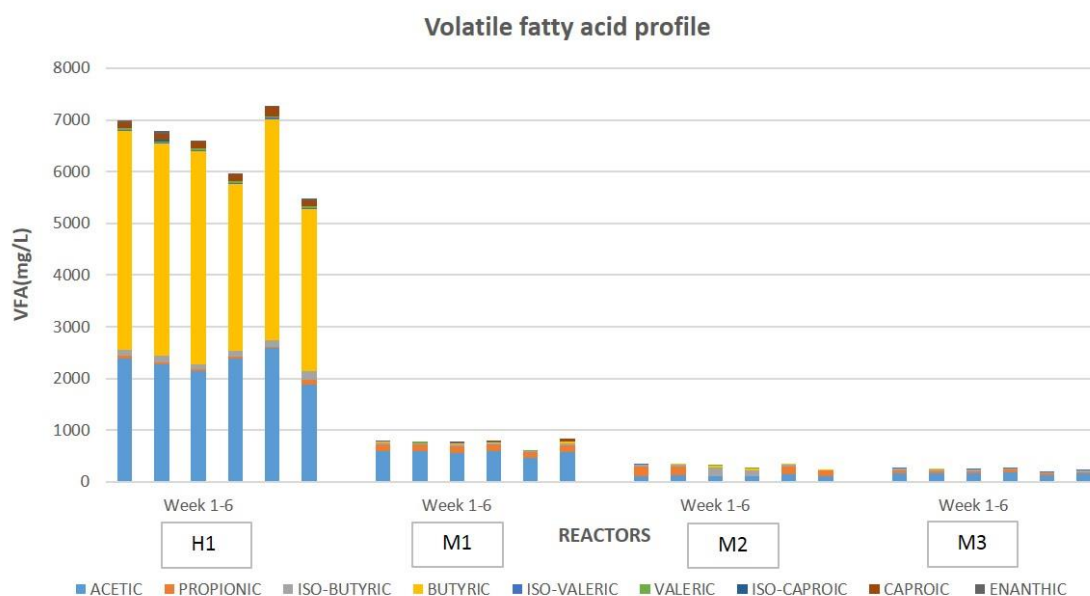
Notes: System A: M₃ (mono-fermentation); system B: H₁+M₁ (two stage fermentation); system C: H₁+M₂ (two stage fermentation).

FOS/TAC: Ratio of volatile fatty acids to total inorganic carbon

TAN: Total ammoniacal nitrogen

S.D: Standard deviation

VFAs produced in the hydrolysis reactor (H₁) are fed as substrates to the methane reactors (M₁ and M₂). The profile of VFA is given in Figure 5.3. It can be observed that the VFA profile for the hydrolysis reactor is dominated by butyric followed by acetic acid. A similar trend in the VFA profile for the hydrolysis of *L. digitata* was observed in batch studies for hydrogen production as reported by Ding et al (2016) and Xia et al (2016).



Notes: System A: M₃ (mono-fermentation); system B: H₁+M₁ (two stage fermentation); system C: H₁+M₂ (two stage fermentation).

Figure 5. 3: Weekly volatile fatty acid profile of the different reactors

The amount of butyric acid was found to be nearly double that of acetic acid. A value of 0.99 g/L of butyric acid as compared to 0.52 g/L of acetic acid was reported by Ding et al. (2016) for co-generation of biohydrogen and biomethane through two-stage batch co-fermentation of macro- and micro-algal biomass. Another recent study done by Xia et al. (2015) show that hydrogen production during hydrolysis of mannitol (carbohydrate present in brown seaweed) generated butyric acid as the dominant VFA. The percentage share of butyric acid of the total soluble metabolic products (SMP) was 62.9% (Xia et al., 2015). Hydrogen production through fermentation of *L. japonica* under different heat treatment conditions by Jung et al. (2011c) showed that 30-45% of VFA was butyric acid followed by acetic acid in the range of 21-35 %. The high concentration of butyric acid was a result of the use of seaweed containing mannitol and the concentrations of the other VFA's were unaffected as the hydrogen in the reactor was not present in high quantities

The VFA profile of the methanogenic reactors follows the normal trend as observed in stable anaerobic digestion processes where the total amount of VFAs is less than 1000 mg/L, which is considered safe for stable operation and better methane yields. However, M₁ shows a value close to this upper range of acceptable total VFA, which might have affected its methane production as it had the lowest methane yields of the three methanogenic reactors.

5.4 Conclusion

A two-stage system including for hydrolytic and methanogenic reactors were commissioned for anaerobic fermentation of the brown seaweed *L. digitata*. This system provided an optimal specific yield of hydrogen (ca. 26 L/kg VS at HRT of 4 d) and methane (ca. 234 L/kg VS at HRT of 14 d), corresponding to an overall energy yield of 8.66 MJ/kg, which is 9.8% higher than the values obtained in a single-stage system (7.89 MJ/kg at HRT of 24 d). The overall HRT can be reduced by 33% whilst improving the energy conversion via two-stage system.

Acknowledgements

This work was funded by Science Foundation Ireland (SFI) through the Centre for Marine and Renewable Energy (MaREI) under Grant No. 12/RC/2302. The work was also co-funded by Gas Networks Ireland (GNI) through the Gas Innovation Group and by ERVIA.

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6.Study of the performance of a thermophilic biological methanation system

Study of the performance of a thermophilic biological methanation system

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Abstract

This study investigated the operation of ex-situ biological methanation at two thermophilic temperatures (55°C and 65°C). Methane composition of 85 to 88% was obtained and volumetric productivities of 0.45 and 0.4 L CH₄ /L reactor were observed at 55°C and 65°C after 24h respectively. It is postulated that at 55°C the process operated as a mixed culture as the residual organic substrates in the starting inoculum were still available. These were consumed prior to the assessment at 65°C; thus the methanogens were now dependent on gaseous substrates CO₂ and H₂. The experiment was repeated at 65°C with fresh inoculum (a mixed culture); methane composition and volumetric productivity of 92% and 0.46 L CH₄ /L reactor were achieved in 24 hours. *Methanothermobacter* species represent likely and resilient candidates for thermophilic biogas upgrading.

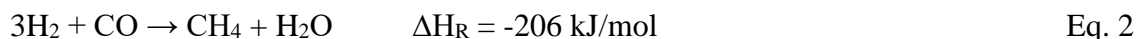
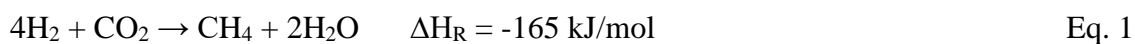
Keywords: Biogas; Power to Gas; Biological Methanation; Methanogenic Archaea; Volatile Fatty Acids.

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6.1 Introduction

Methanation refers to the production of methane through either a catalytic or biological process. The catalytic methanation process proceeds by reacting hydrogen (H₂) with either carbon monoxide (CO) or carbon dioxide (CO₂) to form methane and water. This may be described by Eq. 1 (Sabatier Equation) or by Eq. 2.



The catalytic (Sabatier) process is well understood and has been used for many years in various applications, such as for the removal of trace amounts of carbon oxides in ammonia production. A commonly utilised ammonia synthesis technique is the Haber Bosch process which is operated at an optimal temperature of 500-600 °C (Bicer et al., 2016). A catalyst is required to reduce the activation energy of the reaction and allow it to proceed at higher rates. Such catalysts are typically nickel-based, on an alumina carrier (Charisiou et al., 2016).

Biological methanation is biologically catalysed by methanogenic archaea (Shin et al., 2015). These are strictly anaerobic microbes of the Archaea domain, which carry out the final step in the anaerobic digestion process. Methanogens utilise CO₂, H₂ and acetate as substrates (Nishimura et al., 1992). Most methanogens are capable of utilising H₂ and CO₂ to produce methane, however, only a small number of methanogens can convert acetate to methane. Some, such as those belonging to the genus *Methanosaeta*, may only utilise acetate, while other orders such as *Methanosarcina* are more flexible and can utilise either acetate or H₂ and CO₂. These methanogens generally grow at 35-70°C (Rittmann, 2015; Taubner et al., 2015). The free energy associated with the biological reduction of CO₂ to CH₄ using H₂ is -131 kJ/mol (Madigan, 2012), indicating that the reaction is thermodynamically favourable. Biological methanation may be carried out at industrial scales, typically in conjunction with a conventional biogas plant. The process may be carried out “in-situ” by simply injecting hydrogen into an anaerobic digester containing a variety of anaerobic microorganisms (Luo

and Angelidaki, 2012). Alternatively, it may be carried out “ex-situ” in a separate vessel containing only methanogens (Rittmann et al., 2015).

Large-scale biological methanation is an emerging technology with stirred tank reactors capable of achieving high volumetric productivity and high methane product gas concentration at the same time (Seifert et al., 2014). At lab-scale, various reactor configurations have been trialled with a wide range of results (Bernacchi et al., 2013; Burkhardt et al., 2015; Nishimura et al., 1992; Rachbauer et al., 2016; Rittmann et al., 2012; Seifert et al., 2014). Apart from the physical layout of the reactor, several other process variables are critical. These include temperature, mechanical mixing rates, gas flow rates and the specific strains of methanogens utilised. Mixing is the most energy intensive step, certain mixers like ribbon mixers can consume upto ($10\text{W}/\text{m}^3$), centrifugal mixers with paddle plows ($20\text{W}/\text{m}^3$). A review of the various designs available in the literature is presented in Table 6.1.

Process variables may also vary from one reactor design to another depending on the desired outcome. Certain reactors may be designed to simply enrich the methane content of an existing biogas plant and may aim for a high gas throughput rate rather than high methane concentrations (Bensmann et al., 2014). Other facilities may wish to directly produce a green renewable gas for use as a transport fuel or for gas grid injection, and will thus aim for very high methane concentrations (in excess of 95%) in the product gas (Benjaminsson et al., 2013).

Carbon dioxide and hydrogen can only be consumed by the methanogens at the rate at which they are made available to them in the liquid methanogenic culture. Solubility of hydrogen may be improved by providing a larger transfer surface area such as trickle bed and hollow fibre membrane reactors with packing (see Table 6.1) or by allowing a longer period of time for the transfer to take place through increased retention time (Burkhardt et al., 2015).

Table 6. 1: Existing reactor designs and performance data

Reactor	Temp (°C)	Inoculum	Influent gas	Operation mode	Working volume (L)	Maximum methane concentration (%)	Reference
CSTR	55	Anaerobic digestate	Biogas + H ₂	Continuous	0.6	95.4	(Luo and Angelidaki, 2012)
Trickle bed with packing	37	Anaerobic digestate	H ₂ + CO ₂	Continuous	88	96	(Burkhardt et al., 2015)
Up-flow bed	35	Anaerobic digestate	H ₂ + CO ₂	Continuous	7.8	-	(Lee et al., 2012)
HFM	37	Anaerobic digestate	H ₂ + CO ₂	Continuous	0.195	85	(Lai et al., 2008)
CSTR	37	Anaerobic digestate	H ₂ + CO ₂	Continuous	100	92	(Kim et al., 2013)
CSTR	60	Pure culture	Biogas+ H ₂ H ₂ + CO ₂	Continuous	3	-	(Martin et al., 2013)
Trickle bed reactor	37	Pure culture	H ₂ + CO ₂		58	96	(Rachbauer et al., 2016)
CSTR	65	Pure culture	H ₂ + CO ₂	Continuous	10	85	(Seifert et al., 2014)
Bioreactor with packing	50	Methanogenic culture	H ₂ + CO ₂	Continuous	4	90	(Alitalo et al., 2015)
Closed batch system	55 and 65	Anaerobic digestate	H ₂ + CO ₂	Batch	1.140	92	This study

CSTR: Continuous Stirred Tank Reactor; HFM: Hollow Fibre Membrane Reactor

Where these factors are unable to be altered too severely, such as in the biological methanation process, mechanical mixing may provide an alternative solution such as stirring at high speeds. Mechanical mixing via stirring in a continuously stirred tank reactor (CSTR) is probably the simplest method of assisting H₂ to go into solution. Stirring at speeds of up to 1500 rpm have been demonstrated in lab scale reactors (Bernacchi et al., 2013; Nishimura et

al., 1992; Rittmann et al., 2012; Seifert et al., 2014), however, this is energy intensive when upscaled to commercial reactor scale, where speeds below 60 rpm would be expected. The CSTR may be designed to be tall and narrow, providing a longer path for the gas to rise through and increased contact time with the methanogen culture. Another alternative to mechanical mixing is micro-sparging. In this case, the gas is released into the liquid via micro-porous material, such as a hollow fibre membrane (HFM) (Lai et al., 2008; Lee et al., 2012). This creates very small hydrogen bubbles with high partial pressure and a high ratio of surface area to volume, allowing for more effective hydrogen dissolution. Recirculation of the gas and/or liquid will also assist in the production of a product gas with a high methane content. This concept has been used very effectively in the trickle bed design described by Burkhardt and Busch (Burkhardt and Busch , 2015).

Most of the literature on biological methanation is quiet recent. There are a few studies investigating methanation with pure cultures at thermophilic temperatures and high stirring speeds (Bernacchi et al., 2014). The innovation in this chapter is the detailed study of performance and identification of methanogenic communities in a closed batch system for biological methanation at two thermophilic temperatures, using mixed culture and enriched culture, with different retention times, with H_2 and CO_2 as the influent gases. The objectives of this chapter are to:

- Assess the performance of the system with respect to methane concentration and volumetric productivity with H_2 and CO_2 as the input substrate gases at two different thermophilic temperatures.
- Study the effect of time and temperature on the rate of conversion of the substrate gases to methane.
- Compare the performance of the cultures based on volatile fatty acid profile and identification of methanogens at genus or family level

6.2 Material and Methods

6.2.1 Initial inoculum and nutrient medium

The inoculum for this experiment was sourced from a thermophilic (55°C) reactor treating maize, grass and farmyard manure. The inoculum was stored at 55°C in a water bath until needed, while being fed once a week with cellulose at an organic loading rate (OLR) of 1 kg VS.m⁻³. d⁻¹. As the mixed culture, will only be fed with H₂ and CO₂, it needs to be supplied with certain additional nutrients to maintain growth.

A system for the preparation and dispensing of the anoxic medium was designed, based on guidelines from Wolfe (Wolfe, 2011). The anoxic medium follows the basal medium recipe described by Angelidaki and Sanders (Angelidaki and Sanders, 2004).

6.2.2 Reactor configuration

The reactor consists of a 1 Litre Duran bottle (actual volume 1140 mL). The cap has a rubber seal with two steel pipes drilled in to allow for refreshing of gases and the nutrient medium. A three-way Luer lock stopcock on each pipe provides a simple system for refreshing the gas and anoxic medium, while excluding air from the reactor. Each day, 25 mL of the culture was removed using a syringe (by attaching it to one of the ends of the three-way Luer lock stopcock) and replenished with anoxic medium. This system prevented any gas from entering and leaving the bottles and helpful in pH measurement. The total liquid volume was 380 ml. As the procedure was not carried out over the weekends, the effective HRT was 21 days. At the same time as the medium replenishment, the 760-ml headspace was flushed out with H₂ from a gas bag and 190 mL of carbon dioxide was then injected from a gas-tight syringe to make a 4:1 stoichiometric ratio.

The daily culture samples were analysed for pH level and adjusted to ideally lie between 7.7 and 8.2 as this is generally considered optimal for anaerobic digestion (Laaber, 2011). The ideal pH will vary for different methanogens; for example, Bernacchi and co-workers

obtained high methane production rates between pH 6-7.8 (Bernacchi et al., 2014). The samples were tested for pH using a syringe attached to the three way Luer lock stopcock. The pH range was maintained using 1M hydrochloric acid (HCl) and 3M NaOH. Samples were taken and frozen for future further analysis.

Each day, before refreshing the gases, a 50-mL gas sample was taken from the reactor using a gas tight syringe. This gas sample was then injected into a gas chromatograph (GC) to analyse the product gas makeup. Volumetric productivity was calculated based on the amount of CO₂ injected(0.190L) and the methane composition obtained (% of biogas) per litre of liquid reactor volume(0.380L).

6.2.3 Chemical analyses

Gravimetric measurements including Total Solids (TS) and Volatile Solids (VS) and Volatile Suspended Solids (VSS) were determined by weighing the sample residues that were dried for 24 hours at 105° C and later burning the dried residue at 550° C for 4 hours. Volatile Fatty Acids (VFAs) were determined using a gas chromatograph (Agilent HP 6890 Series, Agilent Technologies, Santa Clara, CA, USA) equipped with a Nukol™ fused silica capillary column (Supelco, Bellefonte, PA, USA), argon as a carrier gas and a flame ionisation detector (Herrmann et al., 2015). Gas samples were measured using a gas chromatograph (Agilent HP 6890 Series, Agilent Technologies, Santa Clara, CA, USA) equipped with a Hayesep R packed column and a thermal conductivity detector. The pH was measured using a Jenway 3510 pH meter.

6.2.4 Reactor start-up and continuous operation of the process.

The VSS of the inoculum was determined before inoculation. The literature indicates that a VSS value of 5-10 g/L should be used for inoculation (Krajete, 2012; Luo and Angelidaki, 2012). For this experiment, 5 g VSS/L was chosen. Three bottles were inoculated with a

mixture of 47.5 mL inoculum and 332.5 mL of anoxic nutrient medium, making up a total of 380 mL. The experiment was conducted in a Thermo Scientific Incubator shaker at an rpm of 180 and initially at a temperature of 55°C. The headspace was replaced with the substrate gases (H₂ and CO₂) batch wise (as this is a closed batch system). The start-up period lasted for about 2 months till relatively stable readings were obtained and it was relatively easy to maintain pH within the range of 7-8 and a methane concentration of at least 80%.

6.2.5 DNA extraction and sequencing

The stages of the process were broken into (A) acclimatisation at 55°C; (B) steady state at 55°C; (C) initial trial at 65°C and (D) reseeded reactor trial at 65°C. Approximately 30ml of suspended solids from each Reactor (1, 2, and 3) for stages B, C and D were centrifuged at 10,000g to pellet biomass (9 samples total). Nucleic acids were extracted in triplicate from these pellets using a CTAB/SDS based lysis buffer and two rounds of phenol-chloroform-isoamyl-alcohol extraction. Primers S-D-Arch-0349-a-S-17 (GYGCASCAGKCGMGAAW) and S-D-Arch-1041-a-A-18 (GGCCATGCACCWCCTCTC) (Klindworth et al., 2012) spanning 16S V3-V6 were selected and appraised using the SILVA testprime database (Klindworth et al., 2012) with parameters of 0 base-pair mismatches, and of 1 base-pair mismatch outside the last 3 3'-base-pairs. Under these constraints, coverage was 70% and 85% for Archaea, 77% and 89% for *Euryarchaeota*, and at least 82%, 75%, 86%, and 100% of the major methanogenic clades (*Methanobacteria*, *Methanomicrobia*, *Methanococci* and *Methanopyri*) respectively. Coverage provided by this primer pair is likely to capture a majority of archaeal sequences. A 692bp product was generated via generic *Taq* polymerase (DreamTaq, ThermoFisher) using a PCR program of initial denaturing for 4min at 94°C; x30 cycles of 1min at 94°C, 54°C, and 72°C each; and a final extension of 4 min at 72°C. Amplicons were purified via gel extraction (QIAGEN) and ligated in EZ-Competent cells (QIAGEN) before being plated on ampicillin; twelve successfully transformed colonies per

Reactor per Stage (108 clones total) were used for M13 PCR before commercial sequencing by GATC (Konstanz, Germany).

6.2.6 Sequence Analysis

Chromatograms were manually curated in FinchTV 1.3.1 (Geospiza Inc.) for read length and accurate base-pair calling (>200bp, PHRED scores ≥ 20). Chimera-checking and OTU (operational taxonomic unit) clustering (<97% identity) were carried out using USEARCH v9.0 (Edgar, 2010). All sequences were submitted to NCBI BLASTn (Altschul et al., 1990) to retrieve 16S reference sequences with closest identities. 16S reference sequences were also retrieved for major methanogenic groups and a bacterial outgroup (*Psychrobacter spcs.*, NR_118027.1). Gapless alignments and Neighbour-Joining phylogenetic trees were generated using MUSCLE v3.8.31 (Edgar, 2004) and formatted in MEGA7 (Kumar et al., 2016). Sequences were uploaded to Genbank under accessions KY077158 - KY077249.

6.3 Results and discussion

6.3.1 Reactor performance at 55°C and 65°C with respect to methane composition, volumetric productivity, retention time and temperature.

The performance of the three reactors were monitored and process variables such as values of methane produced, pH and VFA analysis were actively recorded. Figure 6.1 shows mean and the mean deviation of the weekly values obtained for the triplicate reactors for 24-hour gas sampling. The reactors were operated for 17 weeks at 55°C for the first 12 weeks and at 65°C till week 17.

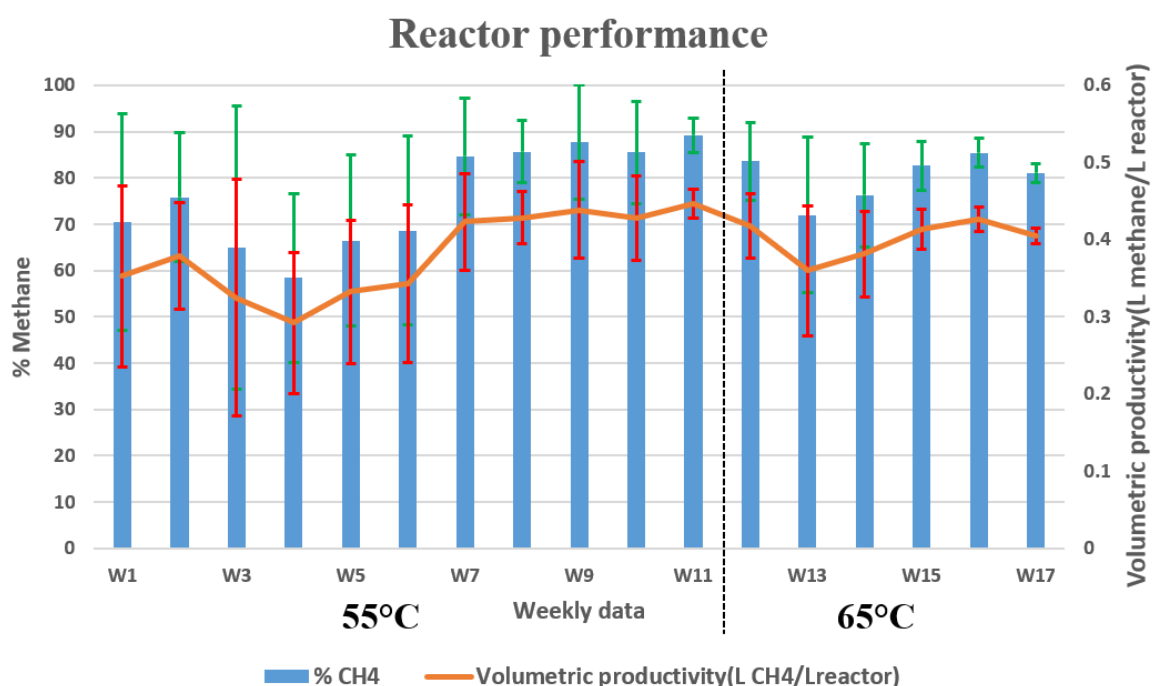


Figure 6. 1: Methane composition and volumetric productivity at 55°C and 65°C for 24-hour retention period

It can be observed that the maximum value for methane composition and methane volumetric productivity were ca. 88% and 0.45(L CH₄ /L reactor) and later dropped to 85% and 0.4(L CH₄ /L reactor) at 65°C for the rest of the time. The first few weeks show the acclimatisation period as the methane composition and volumetric productivities were low. Table 6.2 indicates the performance of the reactors at 12-hour sampling to signify the effect of gas retention time and temperature on methane composition and productivity. The 12-hour gas data at 55°C showed a methane composition and volumetric productivity of 22 % and 0.1(L CH₄ /L reactor) whereas higher values obtained when the reactor was switched to 65 °C with close to 55 % methane composition in the product gas as well as a higher productivity of 0.28(L CH₄ /L reactor). Conducting the experiment at 65°C doubled the methane composition and volumetric productivity for the 12-hour retention period. Luo and Angelidaki showed that the thermophilic (55°C) process is quicker than the mesophilic (37°C) process (Luo and Angelidaki, 2012), but did not investigate any different thermophilic and mesophilic temperatures. Since the inoculum had changed to an enriched culture, methane content saw a

decrease towards the end of the experiment. Mixed digestate has more stability and can perform better under stress as it has a diversity of microbes that can withstand any change or disturbance in their environment as opposed to enriched culture that contain a very narrow spectrum of microbes.

Table 6. 2: Methane composition and volumetric productivities for 12 hour gas sampling at 55°C and 65°C.

	55°C				65°C			
	% Methane	S.D	VP	S.D	% Methane	S.D	VP	S.D
Week 16	21.9	2.63	0.10	0.01	50.29	1.82	0.25	0.8
Week 17	19.8	4.65	0.099	0.02	54.6	5.75	0.27	0.24

S.D: standard deviation

VP: Volumetric productivity (L methane/L reactor) $((0.190 \times 21.9) / (100 \times 0.380))$

6.3.2 Volatile fatty acid profile of the reactors

In an anaerobic digester as the complex compounds are systematically broken down to fatty acids, there is a significant production of predominantly acetic acid followed by other acids. The profile of the VFAs also depends on the particular substrate being broken down. However, in biological methanation processes as there are little breakdown of organic solid or liquid substrates since gaseous compounds are being consumed, very small quantities of VFAs are observed. Figure 6.2 shows the VFAs present in the three reactors.

At 55°C the reactors contained the highest amounts of VFAs and acetic acid; this could be attributed to the initial quantities present in the stock inoculum that were slowly consumed. Although it is hoped that all the CO₂ and H₂ will be consumed directly, an alternative pathway is also possible in which acetate is produced via homoacetogenic microbial activity, in which some of CO₂ and H₂ is converted to acetate (Bensmann et al., 2014; Burak Demirel, 2008; Burkhardt and Busch, 2013; Dahiya and Joseph, 2015; Siriwongrungson et al., 2007). The acetate may then be subsequently converted to CH₄ and CO₂ by acetoclastic methanogens. The quantities of acetate reduced gradually and was probably due to the fact

that there was little acetic acid production after the residual acetic acid in the inoculum was consumed and the only methane production was achieved from gaseous substrates. Residual acetic acid was consumed to form methane and the major contributor to methane production in the later stages of the reaction was the direct reduction of CO₂ by H₂ (Alitalo et al., 2015; Yu and Pinder, 1993).

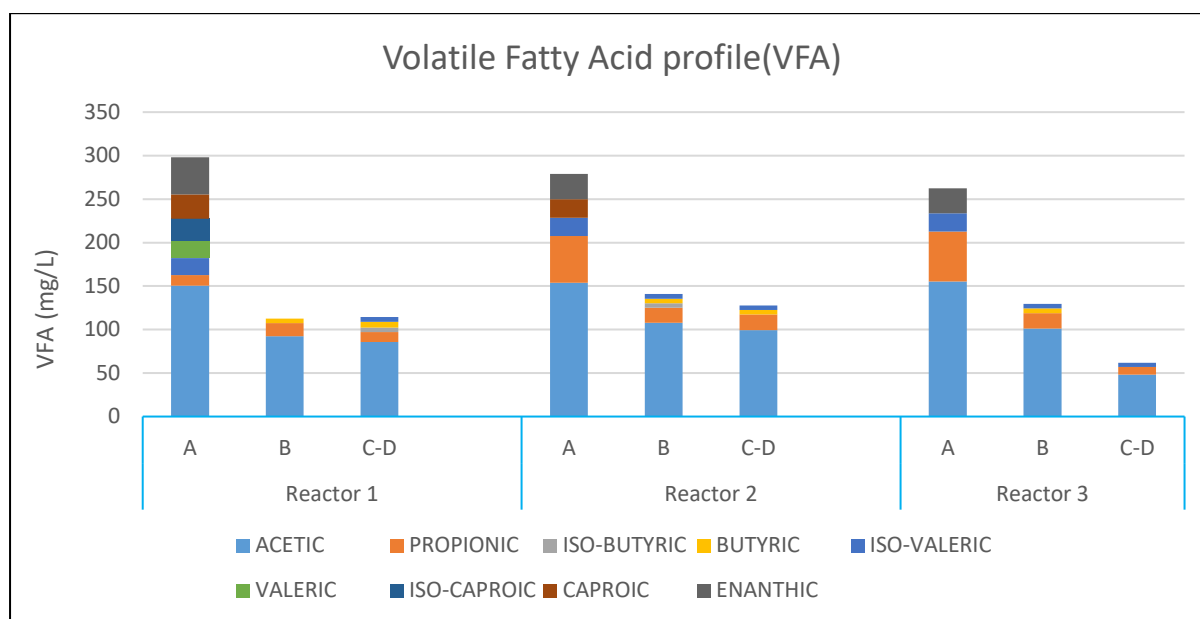


Figure 6. 2: Volatile Fatty Acid profile of the reactors

Note: A- acclimatisation phase at 55°C; B- steady state operation phase at 55°C; C-D is the operation at 65°C

6.3.3 Effect of fresh inoculum on reactor performance

As the performance of the reactors was faster at 65°C, the reactors were re-seeded with fresh stock inoculum and operated at 65°C for 24 hours and 18-hour gas sampling to determine if better and faster methane productivities and composition can be achieved. In the previous experiment, it was observed that 12 hours of biological methanation at 65°C gave nearly 55% methane composition, hence it was decided to observe the methane production at 18 hours along with the 24-hour reading. Figure 6.3 and Table 6.3 highlight the methane production at 65°C with fresh starting stock inoculum. Starting with a fresh inoculum added a few advantages. There was some residual substrate present in the stock inoculum (as the stock

inoculum was fed with cellulose) along with the methanogens and bacteria that are already present in the inoculum. These together along with the gaseous substrates (H_2 and CO_2) seem to give slightly higher methane composition and volumetric productivity of ca. 92% and 0.46(L CH_4 /L reactor) for 24-hour sampling. Higher methane composition and productivity were obtained at 18 hours (77.5% and 0.38 L CH_4 /L reactor) when compared to the 12 hour values obtained in the previous experiment (54.6% and 0.27 L CH_4 /L reactor). It is postulated that this is due to a combination of surplus substrate in the reseeded reactor and the mixed culture of microbes, as well obviously, as the longer retention time. Prolonged use of the stock inoculum leads to a more enriched culture with only the gaseous substrates to feed on. It is suggested by the authors that in a commercial industrial process that reseedling is required to maintain process efficiency.

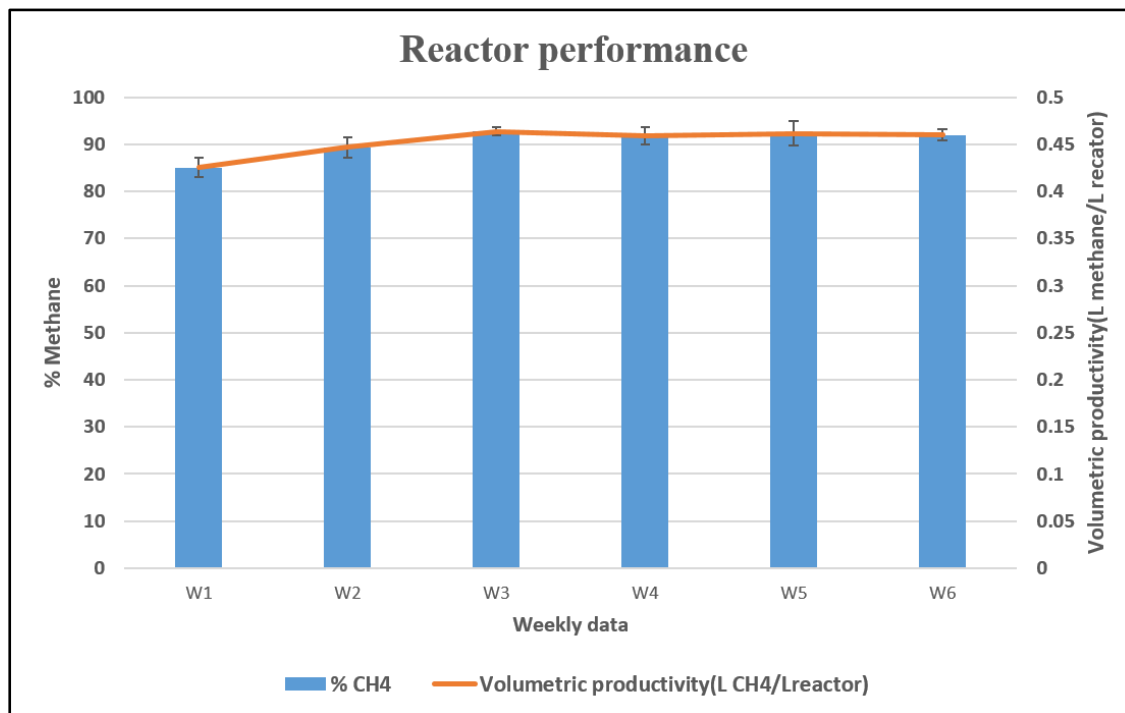


Figure 6. 3: Methane composition and volumetric productivity at 65°C (fresh inoculum) for 24 hours

Table 6. 3: Methane composition and volumetric productivities for 18 hour gas sampling at 65°C.

	65°C			
	% Methane	S.D	VP	S.D
Week 5	77.56	2.52	0.38	0.5
Week 6	75.33	1.66	0.37	0.23

S.D: standard deviation, VP:

Volumetric productivity (L methane/L reactor)

6.3.4 Microbial Community Analysis

Of the 108 clones sequenced, 92 passed quality filters (average length = 626bp), and were clustered at 97% similarity identifying 5 closely-related archaeal OTUs. An OTU table is presented in Table 6.4. Four OTUs aligned at sequences identities >99% with

Table 6. 4: Reference OTUs for sequences clustered at 97% as well as the closest

	Stage B			Stage C			Stage D			
Reactor	R. 1	R. 2	R. 3	R. 1	R. 2	R. 3	R. 1	R. 2	R. 3	Closest Identity
OTU 13B	8	11	10	10	8	6	5	10	10	<i>Methanothermobacter wolfeii</i>
OTU F01	2	1	1	1	0	1	3	1	1	<i>Methanothermobacter wolfeii</i>
OTU B12	0	0	0	0	0	1	1	0	0	<i>Methanothermobacter wolfeii</i>
OTU D04	0	0	0	0	0	0	1	0	0	<i>Methanothermobacter thermautotrophicum</i>
OTU E04	0	0	0	0	0	0	1	0	0	<i>Methanobacterium formicicum</i>

Methanothermobacter wolfeii (OTUs 13B, F01, B12; reference accession KT368944.1) and *Methanothermobacter thermotrophicus* (OTU D04; reference accession HJQ346751.1).

M. wolfeii grows optimally at 55-65°C, pH 7.0-7.7, requiring relatively high concentrations of tungsten (8uM) as a growth factor (Winter et al., 1984). *M. thermautotrophicum* grows optimally between 55-70°C over a pH range of between 7.2-7.6 (Wasserfallen et al., 2000). Both species are capable of growing autotrophically on CO₂ and H₂ and were originally isolated from digester sludges. Additionally, *M. wolfeii* can reduce formate as a carbon source (Winter et al., 1984). A fifth OTU (E04) associated with *Methanobacterium formicicum* Mb9 (accession JN205060.1) at identities >99%. *M. formicicum* can reduce a slightly wider range of carbon sources (CO₂ and formate; 2-propanol and 2-butanol without methanogenesis) but is associated with a much lower temperature range of 37-45°C (Jarvis et al., 2000). A cladogram of sequences from this study, as well as related reference sequences, is provided in (Figure 6.4).

Methanothermobacter-associated OTUs dominate the archaeal community in this thermophilic ex-situ reactor. OTU 13B comprises 85% of all sequences and is evenly distributed across the study, despite a slightly lower abundance in reactors at Stage D. (Figure 4) shows clone sequences clearly cluster with *Methanothermobacter* references, indicating a highly homogeneous archaeal community throughout the trial. Association of OTU E04 with *M. formicicum* suggests closely related taxa at lower abundances. Notably, no sequences align with other methanogenic clades or non-methanogenic Archaea, despite expected coverage of these groups. In particular, a lack of acetoclastic methanogens (Order *Methanosarcinales*) suggests carbon-limited thermophilic conditions may be unsuitable for acetoclasts. The significance of OTUs D04 and E04 is less clear given that they occur only once in this study

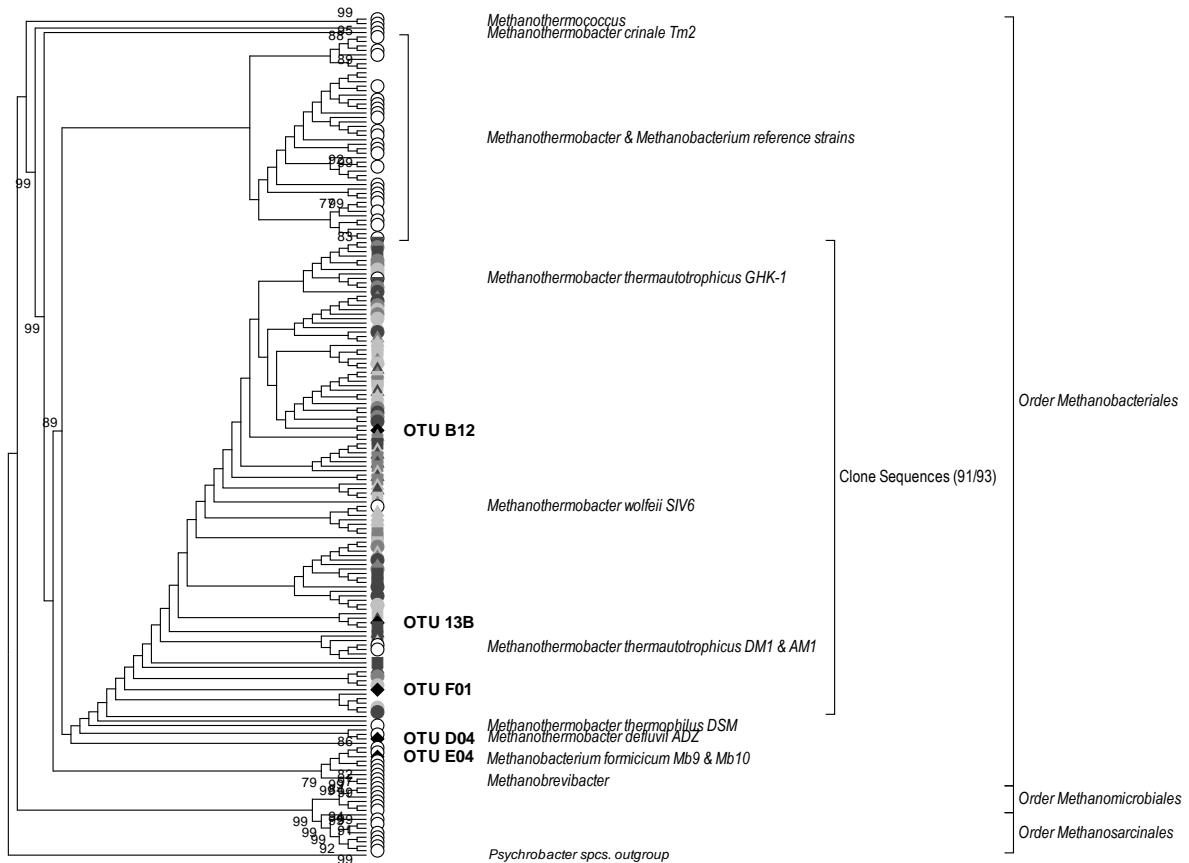


Figure 6. 4: Consensus tree (Neighbour-Joining method with Tamura-Nei distances through 1000 iterations; MEGA) showing evolutionary relationships between cloned and reference sequences in this study.

Note the segregation of Orders *Methanosarcinales* and *Methanomicrobiales* with respect to *O. Methanobacteriales* and clone sequences. The majority of cloned sequences are located among *Methanothermobacter* sequences. Tight clustering with short branch-length reflects the high sequence-similarity of the dataset. No clustering of clones by Reactor or Stage is readily apparent. Only bootstrap values above 75% are presented.

Legend: reference sequences: ○; clustered reference OTUs: ◆; Reactor 1: ●; Reactor 2: ▲; Reactor 3: ■. Stage B: ■; Stage C: ■; Stage D: ■.

6.3.5 Microbial community development

Sampling covered triplicate reactors at 55°C, 65°C, and 65°C with re-inoculation, revealing a homogeneous methanogenic population. Given the changes in reactor conditions (10° increase in temperature, re-inoculation), the consistency of these populations indicates a rapid acclimatisation from the original inoculum community and the stability of those populations

once established. *Methanothermobacter* species therefore represent likely and resilient candidates for thermophilic biogas upgrading.

Re-inoculation of the reactors at Stage D was associated with some recovery of function (from 80-90% to 90-92% CH₄ composition after 24hr) but no significant change in Archaea was observed. It is therefore unlikely that restructuring of methanogen populations had a role in the increased or decreased levels of CH₄. Instead, inoculum may have allowed rescue through the introduction of depleted organic or inorganic materials. Previous studies have identified the importance of trace elements in biogas-orientated in-situ anaerobic digesters (Demirel and Scherer, 2011; Wall et al., 2014) and informed the inclusion of supplements in the reactor media for this ex-situ reactor. Response to further supplementation seen in Stage D may indicate the need for additional growth factors in thermophilic setups - in particular, a requirement for tungsten by *M. wolfeii* (Winter et al., 1984), which associated with over 90% of sequences in this study, may be relevant. Alternatively, a recovery in reactor performance without changes in archaeal taxa may reflect changes in bacterial taxa associated with methanogenic processes in this setup - bacterial taxa excluded at reactor initiation (Stage B, 55°C) may have aided stabilisation when re-inoculated (Stage D, 65°C). Although this study's microbial resolution may be constrained by primer coverage and depth of sequencing, it nevertheless outlines the major methanogenic components of this system through a consistent clustering of sequences. Although some necessary components remain uncharacterised, thermophilic (55°C-65°C) ex-situ biogas upgrading is likely to rely upon select, stable hydrogenotrophic populations of *Methanothermobacter* and *Methanobacterium*

6.4 Conclusion

The operation of an ex-situ biological methanation system is more efficient at 65°C than 55°C. Methane content in excess of 90% can be achieved at volumetric productivity of 0.45 L CH₄/Lreactor/day. As the inoculum ages, it changes from a mixed culture to a more enriched culture; in commercial operations re-seeding of the process would be required. *Methanothermobacter* species dominate the microbial communities in thermophilic ex-situ methanation systems.

Acknowledgements

This work was funded by Science Foundation Ireland (SFI) through the Centre for Marine and Renewable Energy (MaREI) under Grant No. 12/RC/2302. The work was also co-funded by Gas Networks Ireland (GNI) through the Gas Innovation Group and by ERVIA.

The reviewers of the paper have greatly enhanced the quality of this paper through rigorous interrogation of the original manuscript and expanded the remit to include description of the microbial community.

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7. Conclusions and Recommendations

7.1 Conclusions

A perspective on gaseous biofuel production from micro-algae generated from CO₂ from a coal-fired power plant:

- A 1 GW_e coal power plant (at 35% efficiency and capacity factor of 75%) consumes around 2.72Mt of coal per annum whilst producing 6.77Mt of CO₂. The emission from such a plant can be captured by micro-algae at an efficiency of 50 and 80% for open and closed cultivation systems respectively. The total amount of micro-algal volatile solids (VS) produced would be 2.69Mt and 1.68Mt for open and closed systems.
- Photo bioreactors (closed system) have higher productivities and occupy lesser area when compared to raceway ponds (open system). If such systems are to be used for cultivation of micro-algae from coal powered power plants, then an area of 52,303 ha would be required by raceway ponds when compared to 19,192 ha required by tubular photo bioreactors.
- The micro-algae thus produced is subjected to sequential dark and photo fermentation to produce hydrogen and volatile fatty acids. This is followed by methanation to produce methane. Upon the application of such a three-phase system, one tonne of micro-algal VS can produce 8.8 GJ of renewable gas. Hence a 1 GW_e coal plant with carbon capture by micro-algae can produce 23.7 PJ of energy.
- Techno-economic analysis from the literature of tubular photo bioreactors reveals that the operation cost of such reactors is very high as they have high energy requirements (such as pumping, illumination etc.). Such systems utilise more energy for operation than the actual energy produced by the micro-algal biomass.

Seaweed Biofuel Derived from Integrated Multi-Trophic Aquaculture

- Seaweed (macro-algae) has been used as food in some Asian countries. It has several industrial applications in the field of hydrocolloids such as emulsifiers, gelling agents and production of bio-plastics. Natural stocks cannot keep up with the demand and can be quite detrimental to the environment if they are depleted. Hence close to 90% of seaweed used today comes from aquaculture. The total value of the seaweed industry per annum is US\$5.5-6 billion, with human consumption accounting for US\$ 5 billion. Whereas the hydrocolloids industry was estimated to be worth US\$ 600 million in 2003 and has increased to US\$ 1156 million in 2014.
- Consumption of meat protein has steadily increased around the world. Protein derived from fish contributed 16.7% to the global animal protein intake in 2010, about 150 g of fish can cater for more than half of an adult's daily protein requirement. Salmon trade (both wild and cultivated) has increased considerably and contributes 14% to world fishery trade.
- Farmed salmon contributes 60% to global salmon production. Hence environmental precautions need to be taken to prevent eutrophication of water caused by the release of the wastes from fish farms. Integrated multi-trophic aquaculture can be used to sequester these wastes and produce seaweed at the same time.
- If 1.25 % of energy in transport is to be provided by seaweed, then under the IMTA system 13Mt of salmon would be required to produce 168Mt of seaweed (*S. latissima*) that can be digested in 2603 coastal digesters.

Comparative study of single and two stage mono-fermentation of brown seaweed *Laminaria digitata*

- Brown seaweed *Laminaria digitata* was subjected to single and two stage fermentation. There were two systems performing as two stage fermentation with different retention times of 24d ($H_1:M_1$) and 18d ($H_1:M_2$). The methane yield of the two systems were 176 and 234 L/kg VS respectively. The second two stage system ($H_1:M_2$) had a higher loading rate than the first system ($H_1:M_1$), hence higher methane yields were observed.
- A single stage system was also run simultaneously with a retention time of 24d and a methane yield of 221 L/kg VS. This value is higher than the two-stage system of ($H_1:M_1$) but on par with the other system ($H_1:M_2$) as the single stage system was however more stable than the two-stage systems.
- Higher methane compositions of 58% and 61% were obtained for both the two stage systems when compared to the single stage system (50%). Energy yields of 7.89 MJ/kg VS (single stage system) and 8.66 MJ/kg VS (two stage 18 d ($H_1:M_2$) system) were obtained, which is 9.8 % higher than the single stage system. The overall energy yield of the 24d two stage ($H_1:M_1$) system was comparatively lower (6.57 MJ/kg VS).
- The hydrogen yield of the hydrolysis reactor was on par with that found in literature for two stage continuous systems. A hydrogen yield of ca.26 L/kg VS with butyric acid being the dominant VFA was obtained.

Study of the performance of a thermophilic biological methanation system

- Biological methanation was carried out at 55°C starting with mixed culture as inoculum and was later operated at 65°C. The methane composition obtained for 24h was in the range of 85-88% with volumetric productivities of 0.45 and 0.4LCH₄/Lreactor.
- However, after running it for a long period, most of the residual acetic acid was consumed and the microbes had to subsist only on the gaseous substrates (H₂ and CO₂) to produce methane as a result a lower methane composition was observed in the latter stages. It can be hypothesised that the mixed culture had become an enriched culture now.
- The experiment was started again with fresh mixed culture as inoculum at 65°C, methane composition of 92% and volumetric productivity of 0.46LCH₄/Lreactor were observed at 24h. *Methanothermobacter* species were identified as predominant and to represent the most likely resilient candidates for thermophilic biogas upgrading.

7.2 Recommendations

Practicalities of implementing third generation gaseous biofuels:

Micro-algal production is the main hindrance to its technology as it is more expensive than growing terrestrial crops. To obtain high yields closed systems (photo-bioreactors) need to be used. High level of instrumentation to maintain light intensity, efficient uptake of CO₂, dissolution of nutrient medium (inorganic salts containing nitrogen, phosphorus and iron), pumping energy required for efficient mixing of the reactors are the steps that are expensive.

However, if raceway ponds are used the cost of instrumentation can be reduced but it results in low yield of algae and occupies 3 times the space required by photobioreactors. Such large land footprint nullifies the benefit of low cost of production. In such a scenario arable land could be used to produce algae which will interfere with the production of food crops thus again leading to a food-fuel debate. One possible solution is to use offshore algal ponds without harming the coastal environment. Cost of offshore open and closed systems of algal production should be investigated. Apart from the cost and energy input a separate study needs to be done to investigate variation in performance of the specie which is a function of its geographical conditions, diurnal variation in the amount of carbon and light that it receives, effect of nutrient availability. These are the factors that affect the biomass yield of algae. Future research should include detail analysis of parasitic energy demand, cost of nutrient addition and cost of construction at such a large scale.

Macro-algal/ seaweed production depends on aquaculture to a large extent as natural sources are not enough to cater to the demand. Hence, they get depleted faster affecting the coastal environment as certain aquatic species use it as food. Farmed seaweed can also be consumed as food by humans if it is of food grade quality as it can fetch higher price as food and to produce hydrocolloids rather than feedstock for biofuel production. Cost of offshore seaweed farms need to be studied as the method of cultivation (long line net cultivation or floating method) , cost of harvesting , infestation by other species , direction of water currents affect the yield obtained. Size and cost of such offshore farms and coastal digesters need to be studied

Non-biological source of third generation gaseous biofuel comes from biological methanation. Cheap and surplus sources of hydrogen and carbon dioxide are the most important requirements for this technology. Cost of producing hydrogen via electrolysis is the most important step and is expensive. Different methods of electrolysis (polymer electrolyte

membrane, alkaline , and solid oxide electrolyzers) are being studied for their yield of hydrogen and cost of production. Efficient biological methanation depends on strict pH and temperature control along with efficient hydrogen dissolution needs a good degree of instrumentation. Cost of such reactors, energy for mixing can make the operation of ex-situ biological methanation expensive. The size of such reactors and its loading rate along with a detailed comparative cost analysis between ex-situ and in-situ biological methanation should form the next part this study.

This thesis demonstrates that third generation gaseous biofuel can be derived from algal and non-biological sources. Micro-algae can be grown using carbon emissions from industries. The resultant biomass can then be anaerobically digested to produce methane. However, it is recommended that the cost of operation and energy input of photo-bioreactors should be studied extensively to keep the technology viable. This thesis only gives a broad idea about this technology and should be investigated further as discussed.

Other algal biomass such as seaweed can be grown using waste nitrogenous streams from fish farms. This method is more economical than the former; however, if producing hydrocolloids from seaweed is more profitable than biogas production, the price of this feedstock may become too high and uneconomical for biogas production. This is an ongoing debate as to whether seaweed should be used for biofuel or should be used in other industries.

Cost of biogas production can be reduced by implementing two stage fermentation that allows for lesser hydraulic retention times and higher organic loading rates can be used for higher and faster methane yields. Future work can be carried out on the cost analysis of two stage versus single stage operation. Trace element addition (nutrient addition) in the latter stages of the operation should be studied in detail.

Biological methanation of hydrogen (from non-biological sources) and carbon dioxide can be carried out to produce methane compositions in excess of 90%. Hydrogen and carbon dioxide should come from surplus or cheap sources to make the technology economically feasible. As the culture got more enriched with specific thermophilic specie the stability of the reactor reduced; it can be inferred that with enriched culture better and sophisticated processes and instrumentation should be used to avoid contamination and to maintain or even increase process efficiency. Such methods will increase the cost of operation and this should be further studied.

Thus, this thesis provides a study on the use and scope of third generation gaseous biofuels from algae and non-biological sources. This is in line with the latest developments in the field of biofuel that is trying to move away from first and second-generation feedstock that have been heavily criticised for its indirect land usage and its role in causing inflation of food prices. Algae and non-biological sources of gaseous biofuels should be implemented after a thorough study of their techno-economic feasibility.

Appendix A: Co-authored papers on third generation feedstock and two stage fermentation

Assessment of increasing loading rate on two-stage digestion of food waste

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Abstract

A two-stage food waste digestion system involved a first stage hydrolysis reactor followed by a second stage methanogenic reactor. Organic loading rates (OLR) were increased from 6 to 15 g VS L⁻¹ d⁻¹ in the hydrolysis reactor and from 2 to 5 g VS L⁻¹ d⁻¹ in the methanogenic reactor. The retention time was fixed at 4 days (hydrolysis reactor) and 12 days (methane reactor). A single-stage digester was subjected to similar loading rates as the methanogenic reactor at 16 days retention. Increased OLR resulted in higher quantities of liquid fermentation products from the first stage hydrolysis reactor. Solubilisation of chemical oxygen demand peaked at 47% at the maximum loading. However, enhanced hydrolysis yields had no significant impact on the specific methane yields. The two-stage system increased methane yields up to 23% and enriched methane content by an average of 14% to levels of 71%.

Keywords: Two-stage digestion; Food waste; Hydrolysis; Biogas; High performance reactors

Highlights:

- Single and two-stage digestion of food waste was compared at increased loading.
- The methane content of the biogas increased by 14% to 71% in the two-stage system.
- The two-stage system yielded up to 23% more methane than the single-stage system.
- The two-stage system produced up to 404 L CH₄ kg⁻¹ VS or 15.1 MJ kg VS⁻¹.

Fermentative bio-hydrogen production from galactose

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Abstract

Bio-hydrogen production through fermentation of waste biomass has considerable benefits both as a waste treatment process and a substitute for fossil fuels. Galactose, which can be the dominant component in various biomass wastes (such as marine red algae, cheese and dairy industry waste streams) was fermented by anaerobic fermentative bacteria to assess bio-hydrogen production. The impacts of pH, the YE/G (yeast extract/galactose) ratio and substrate concentration were investigated and optimised by response surface methodology. Hydrogen production was mainly via acetic and butyric acid pathways, while hydrogen consumption was via caproic acid and homoacetogenesis pathways. The hydrogen yield and production rate were improved to 278.1 mL/g galactose (2.23 mol/mol galactose) and 33.6 mL/g galactose/h, respectively, under the optimal conditions (pH value of 6.05, YE/G ratio of 0.56 and substrate concentration of 5 g volatile solid/L). The overall energy conversion efficiency from substrates to hydrogen and soluble metabolic products reached 68.6%.

Keywords: Galactose; Bio-hydrogen; Fermentation; Soluble metabolic products; Energy conversion efficiency

Highlights:

- Galactose was fermented by anaerobic fermentative bacteria for H₂ production.
- H₂ yield of galactose was improved by response surface methodology.
- The maximal H₂ yield achieved 278.1 mL/g galactose (2.23 mol/mol galactose). The maximal H₂ production rate achieved 33.6 mL/g galactose/h.
- Overall energy production efficiency reached 68.6% via fermentation.

Production of hydrogen, ethanol and volatile fatty acids from the seaweed carbohydrate mannitol

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Abstract

Fermentative hydrogen from seaweed is a potential biofuel of the future. Mannitol, which is a typical carbohydrate component of seaweed, was used as a substrate for hydrogen fermentation. The theoretical specific hydrogen yield (SHY) of mannitol was calculated as 5 mol H₂/mol mannitol (615.4 mL H₂/g mannitol) for acetic acid pathway, 3 mol H₂/mol mannitol (369.2 mL H₂/g mannitol) for butyric acid pathway and 1 mol H₂/mol mannitol (123.1 mL H₂/g mannitol) for lactic acid and ethanol pathways. An optimal SHY of 1.82 mol H₂/mol mannitol (224.2 mL H₂/g mannitol) was obtained by heat pre-treated anaerobic digestion sludge under an initial pH of 8.0, NH₄Cl concentration of 25 mM, NaCl concentration of 50 mM and mannitol concentration of 10 g/L. The overall energy conversion efficiency achieved was 96.1%. The energy was contained in the end products, hydrogen (17.2%), butyric acid (38.3%) and ethanol (34.2%).

Keywords: Seaweed; Mannitol; Hydrogen; Ethanol; Biofuels

Highlights

- Mannitol can be efficiently fermented by AFB to produce H₂ and SMPs.
- The theoretical maximum specific H₂ yield is 615.4 mL H₂/g mannitol (5 mol/mol).
- The optimal specific H₂ yield achieved was 224.2 mL H₂/g mannitol (1.82 mol/mol).
- The overall energy conversion efficiency achieved was 96.1% via fermentation.
- Energy production was dominated by H₂ (17%), butyric acid (38%) and ethanol (34%).

Production of hydrogen, ethanol and volatile fatty acids through co-fermentation of macro- and micro-algae

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Abstract

Algae may be fermented to produce hydrogen. However micro-algae (such as *Arthrospira platensis*) are rich in proteins and have a low carbon/nitrogen (C/N) ratio, which is not ideal for hydrogen fermentation. Co-fermentation with macro-algae (such as *Laminaria digitata*), which are rich in carbohydrates with a high (C/N) ratio, improves the performance of hydrogen production. Algal biomass, pre-treated with 2.5% dilute H₂SO₄ at 135 °C for 15 min, effected a total yield of carbohydrate monomers (CMs) of 0.268 g/g volatile solids (VS). The CMs were dominating by glucose and mannitol and most (ca. 95%) were consumed by anaerobic fermentative micro-organisms during subsequent fermentation. An optimal specific hydrogen yield (SHY) of 85.0 mL/g VS was obtained at an algal C/N ratio of 26.2 and an algal concentration of 20 g VS/L. The overall energy conversion efficiency increased from 31.3% to 54.5% with decreasing algal concentration from 40 to 5 VS g/L.

Keywords: Algae; Fermentation; Hydrogen; Volatile fatty acids; Ethanol

Highlights

- Micro- and macro-algae may be fermented to produce H₂ and ethanol.
- The optimal pre-treatment was steam heating at 135 °C with 2.5% H₂SO₄.
- An optimal H₂ yield of 85.0 mL/g VS was achieved at a C/N ratio of 26.2.
- The overall energy conversion efficiency reached 54.5% by fermentation.
- Energy in algae was converted to H₂ (5.7%), ethanol (15.6%) and VFAs (33.2%).